REFERENCE 2: 106:110407

=> fil medl,caplus,biosis,embase,wpids;s sm22?
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 8.13 9.48

FILE 'MEDLINE' ENTERED AT 14:03:05 ON 19 JUN 2000

FILE 'CAPLUS' ENTERED AT 14:03:05 ON 19 JUN 2000
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2000 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 14:03:05 ON 19 JUN 2000
COPYRIGHT (C) 2000 BIOSIS(R)

FILE 'EMBASE' ENTERED AT 14:03:05 ON 19 JUN 2000
COPYRIGHT (C) 2000 Elsevier Science B.V. All rights reserved.

FILE 'WPIDS' ENTERED AT 14:03:05 ON 19 JUN 2000
COPYRIGHT (C) 2000 DERWENT INFORMATION LTD

L2 87 FILE MEDLINE
L3 105 FILE CAPLUS
L4 105 FILE BIOSIS
L5 85 FILE EMBASE
L6 11 FILE WPIDS

TOTAL FOR ALL FILES
L7 393 SM22?

=> s latent(l)((transform? growth factor or tgf)(w)(b or beta) or d11.303.900.720/ct or d12.644.900.720/ct or d24.185.348.900.720/ct or d24.611.350.400.800/ct)
L8 638 FILE MEDLINE
L9 633 FILE CAPLUS
L10 666 FILE BIOSIS
L11 611 FILE EMBASE
L12 19 FILE WPIDS

TOTAL FOR ALL FILES
L13 2567 LATENT(L)((TRANSFORM? GROWTH FACTOR OR TGF)(W)(B OR BETA) OR D11.303.900.720/CT OR D12.644.900.720/CT OR D24.185.348.900.720/CT OR D24.611.350.400.800/CT)

=> s l13(5a)(bind? protein? or inhibitor?)
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L8 (5A)(BIND?'
L14 336 FILE MEDLINE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L9 (5A)(BIND?'
L15 304 FILE CAPLUS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L10(5A)(BIND?'
L16 314 FILE BIOSIS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH

```
FIELD CODE - 'AND' OPERATOR ASSUMED 'L11(5A)(BIND?'
L17         296 FILE EMBASE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L12(5A)(BIND?'
L18           7 FILE WPIDS

TOTAL FOR ALL FILES
L19        1257 L13(5A)(BIND? PROTEIN? OR INHIBITOR?)

=> s (integrin? or d12.776.543.750.705.408/ct or d24.611.834.408/ct)(l)link?
kinase?
L20          27 FILE MEDLINE
L21          36 FILE CAPLUS
L22          47 FILE BIOSIS
L23          26 FILE EMBASE
L24           3 FILE WPIDS

TOTAL FOR ALL FILES
L25         139 (INTEGRIN? OR D12.776.543.750.705.408/CT OR
D24.611.834.408/CT)(
                L) LINK? KINASE?

=> s lef 1(5a)(b or beta)(w)catenin?
L26          18 FILE MEDLINE
L27          25 FILE CAPLUS
L28          16 FILE BIOSIS
L29          17 FILE EMBASE
L30           2 FILE WPIDS

TOTAL FOR ALL FILES
L31          78 LEF 1(5A)(B OR BETA)(W) CATENIN?

=> s (neural crest or a16.254.600/ct or neuroblastoma? or
c4.557.465.625.600.590.650.550/ct or c4.557.580.625.600.590.650.550/ct or
c4.557.470.670.590.650.550/ct)
L32       21269 FILE MEDLINE
L33       11862 FILE CAPLUS
L34       21237 FILE BIOSIS
L35       18972 FILE EMBASE
L36         465 FILE WPIDS

TOTAL FOR ALL FILES
L37       73805 (NEURAL CREST OR A16.254.600/CT OR NEUROBLASTOMA? OR
C4.557.465.
                625.600.590.650.550/CT OR C4.557.580.625.600.590.650.550/CT OR
                C4.557.470.670.590.650.550/CT)

=> s l37(l)(smooth(1a)muscle? or muscle? or a2.633.570/ct or a10.690.467/ct)
L38         521 FILE MEDLINE
L39         341 FILE CAPLUS
L40         608 FILE BIOSIS
L41         471 FILE EMBASE
L42          26 FILE WPIDS

TOTAL FOR ALL FILES
L43        1967 L37(L)(SMOOTH(1A) MUSCLE? OR MUSCLE? OR A2.633.570/CT OR
A10.690
                .467/CT)

=> s l43(l)differ?
L44         238 FILE MEDLINE
```

```
L45         146 FILE CAPLUS
L46         229 FILE BIOSIS
L47         223 FILE EMBASE
L48           5 FILE WPIDS

TOTAL FOR ALL FILES
L49         841 L43(L) DIFFER?

=> s l49(l)(g5.331.375/ct or g5.331.370/ct or phenotyp? or
gene(2a)regulat?(2a)express?)
L50          31 FILE MEDLINE
L51          15 FILE CAPLUS
L52          31 FILE BIOSIS
L53          30 FILE EMBASE
L54           0 FILE WPIDS

TOTAL FOR ALL FILES
L55         107 L49(L)(G5.331.375/CT OR G5.331.370/CT OR PHENOTYP? OR GENE(2A)
                REGULAT?(2A) EXPRESS?)

=> s l7(l)l55
L56           1 FILE MEDLINE
L57           1 FILE CAPLUS
L58           1 FILE BIOSIS
L59           1 FILE EMBASE
L60           0 FILE WPIDS

TOTAL FOR ALL FILES
L61           4 L7(L) L55

=> dup rem l61
PROCESSING COMPLETED FOR L61
L62             1 DUP REM L61 (3 DUPLICATES REMOVED)

=> d cbib abs hit

L62  ANSWER 1 OF 1  MEDLINE                                  DUPLICATE 1
1998165765  Document Number: 98165765.   In vitro system for differentiating
     pluripotent neural crest cells into smooth muscle cells. Jain M K; Layne
M
     D; Watanabe M; Chin M T; Feinberg M W; Sibinga N E; Hsieh C M; Yet S F;
     Stemple D L; Lee M E. (Cardiovascular Biology Laboratory, Harvard School
     of Public Health, Boston, Massachusetts 02115, USA. ) JOURNAL OF
     BIOLOGICAL CHEMISTRY, (1998 Mar 13) 273 (11) 5993-6. Journal code: HIV.
     ISSN: 0021-9258. Pub. country: United States. Language: English.
AB   The change in vascular smooth muscle cells (SMC) from
     a differentiated to a dedifferentiated state is the critical
     phenotypic response that promotes occlusive arteriosclerotic
     disease. Despite its importance, research into molecular mechanisms
     regulating smooth muscle differentiation has
     been hindered by the lack of an in vitro cell differentiation
     system. We identified culture conditions that promote efficient
     differentiation of Monc-1 pluripotent neural
     crest cells into SMC. Exclusive Monc-1 to SMC
     differentiation was indicated by cellular morphology and
     time-dependent induction of the SMC markers smooth
     muscle alpha-actin, smooth muscle myosin heavy
     chain, calponin, SM22alpha, and APEG-1. The activity of the
     SM22alpha promoter was low in Monc-1 cells.
     Differentiation of these cells into SMC caused a 20-30-fold
     increase in the activity of the wild-type SM22alpha promoter and
```

that of a hybrid promoter containing three copies of the CArG element. By gel mobility shift analysis, we identified new DNA-protein complexes in nuclear extracts prepared from **differentiated** Monc-1 cells. One of the new complexes contained serum response factor. This Monc-1 to SMC model should facilitate the identification of nodal regulators of **smooth muscle** development and **differentiation**.

AB The change in vascular **smooth muscle** cells (SMC) from a **differentiated** to a dedifferentiated state is the critical **phenotypic** response that promotes occlusive arteriosclerotic disease. Despite its importance, research into molecular mechanisms regulating **smooth muscle differentiation** has been hindered by the lack of an in vitro cell **differentiation** system. We identified culture conditions that promote efficient **differentiation** of Monc-1 pluripotent **neural crest** cells into SMC. Exclusive Monc-1 to SMC **differentiation** was indicated by cellular morphology and time-dependent induction of the SMC markers **smooth muscle** alpha-actin, **smooth muscle** myosin heavy chain, calponin, **SM22alpha**, and APEG-1. The activity of the **SM22alpha** promoter was low in Monc-1 cells. **Differentiation** of these cells into SMC caused a 20-30-fold increase in the activity of the wild-type **SM22alpha** promoter and that of a hybrid promoter containing three copies of the CArG element. By gel mobility shift analysis, we identified new DNA-protein complexes in nuclear extracts prepared from **differentiated** Monc-1 cells. One of the new complexes contained serum response factor. This Monc-1 to SMC model should facilitate the identification of nodal regulators of **smooth muscle** development and **differentiation**.

```
=> s sm22alpha
L63          25 FILE MEDLINE
L64          17 FILE CAPLUS
L65          30 FILE BIOSIS
L66           2 FILE EMBASE
L67           3 FILE WPIDS

TOTAL FOR ALL FILES
L68          77 SM22ALPHA

=> s l68 not l61
L69          24 FILE MEDLINE
L70          17 FILE CAPLUS
L71          29 FILE BIOSIS
L72           2 FILE EMBASE
L73           3 FILE WPIDS

TOTAL FOR ALL FILES
L74          75 L68 NOT L61

=> dup rem l74
PROCESSING COMPLETED FOR L74
L75          46 DUP REM L74 (29 DUPLICATES REMOVED)

=> d 1-46 cbib abs

L75  ANSWER 1 OF 46  WPIDS COPYRIGHT 2000   DERWENT INFORMATION LTD
AN   2000-126315 [11]   WPIDS
CR   1999-023458 [02]
AB   US   6015711 A UPAB: 20000301
     NOVELTY - Expressing foreign DNA in mammalian arterial smooth-muscle
cells
```

comprises introducing into the cells a gene transfer vector comprising a mouse SM22 alpha promoter operably linked to foreign DNA encoding a desired polypeptide or RNA molecule, where the promoter has one of four defined sequences given in the specification.

ACTIVITY - Vasotropic.

No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - The method can be used to express therapeutic polypeptides, RNA molecules or reporter molecules in mammalian arterial smooth muscle, especially therapeutic polypeptides selected from basic fibroblast growth factor 1 receptor dominant negative mutant, p21, angiopeptin, endothelial cell nitric oxide synthase and non-phosphorylated Rb, therapeutic RNA molecules selected from antisense c-myc RNA and antisense c-myb RNA, or reporter molecules selected from beta -galactosidase, luciferase and chloramphenicol acetyltransferase (all claimed). Expression of the polypeptides and antisense RNA can be used for the treatment of vascular diseases e.g. restenosis of the vascular wall following balloon angioplasty.

ADVANTAGE - The SM22 alpha promoter is active only in the smooth-muscle cells of large arteries and their immediate branches, and not in small arteries, veins or visceral organs, making it suitable for gene therapy of the arterial wall without leaky expression in other tissues. SM22 alpha is one of the only smooth muscle cell markers which is not down regulated during vascular occlusive disease.

Dwg.0/6

L75 ANSWER 2 OF 46 MEDLINE DUPLICATE 1

2000230168 Document Number: 20230168. Fibroblast growth factor plays a critical role in **SM22alpha** expression during Xenopus embryogenesis. Oka T; Shiojima I; Monzen K; Kudoh S; Hiroi Y; Shiokawa K; Asashima M; Nagai R; Yazaki Y; Komuro I. (Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Japan.) ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, (2000 Apr) 20 (4) 907-14. Journal code: B89. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB Although smooth muscle cells (SMCs) are critical components of the circulatory system, the regulatory mechanisms of SMC differentiation remain largely unknown. In the present study, we examined the mechanism of SMC differentiation by using Xenopus laevis **SM22alpha** (XSM22alpha) as an SMC-specific marker. XSM22alpha cDNA contained a 600-bp open reading frame, and the predicted amino acid sequences were highly conserved in evolution. XSM22alpha transcripts were first detected in heart anlage, head mesenchyme, and the dorsal side of the lateral plate mesoderm at the tail-bud stage, possibly representing the precursors of muscle lineage. At the tadpole stage, XSM22alpha transcripts were restricted to the vascular and visceral SMCs. XSM22alpha was strongly induced by basic fibroblast growth factor (FGF) in animal caps. Although expressions of Xenopus cardiac actin were not affected by the expression of a dominant-negative FGF receptor, its injection dramatically suppressed the XSM22alpha expression. These results suggest that XSM22alpha is a useful molecular marker for the SMC lineage in Xenopus and that FGF signaling plays an important role in the induction of XSM22alpha and in the differentiation of SMCs.

L75 ANSWER 3 OF 46 MEDLINE DUPLICATE 2

2000115087 Document Number: 20115087. Mouse BTEB3, a new member of the basic

transcription element binding protein (BTEB) family, activates expression from GC-rich minimal promoter regions. Martin K M; Cooper W N; Metcalfe J C; Kemp P R. (Section of Cardiovascular Biology, Department of Biochemistry, Cambridge University, Tennis Court Road, Cambridge CB2 1QW, U.K.) BIOCHEMICAL JOURNAL, (2000 Feb 1) 345 Pt 3 529-33. Journal code: 9YO. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Members of the three-zinc-finger family of transcription factors play an important role in determining basal transcription. We have cloned mouse BTEB3 (mBTEB3), a new member of the basic transcription element binding protein (BTEB) family, which is expressed in a wide variety of tissues. mBTEB3 activates transcription of the simian virus 40 early promoter (4-fold) and of the tissue-specific **SM22alpha** promoter (100-fold), suggesting that, like BTEB1 and Sp1, mBTEB3 is a basal transcription factor.

L75 ANSWER 4 OF 46 MEDLINE DUPLICATE 3

2000115076 Document Number: 20115076. Four isoforms of serum response factor that increase or inhibit smooth-muscle-specific promoter activity. Kemp P R; Metcalfe J C. (Section of Cardiovascular Biology, Department of Biochemistry, University of Cambridge, The Downing Site, Tennis Court Road, Cambridge CB2 1QW, U.K.. pk@mole.bio.cam.ac.uk) . BIOCHEMICAL JOURNAL, (2000 Feb 1) 345 Pt 3 445-51. Journal code: 9YO. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Serum response factor (SRF) is a key transcriptional activator of the c-fos gene and of muscle-specific gene expression. We have identified four forms of the SRF coding sequence, SRF-L (the previously identified form), SRF-M, SRF-S and SRF-I, that are produced by alternative splicing. The new forms of SRF lack regions of the C-terminal transactivation domain by splicing out of exon 5 (SRF-M), exons 4 and 5 (SRF-S) and exons 3, 4 and 5 (SRF-I). SRF-M is expressed at similar levels to SRF-L in differentiated vascular smooth-muscle cells and skeletal-muscle cells, whereas SRF-L is the predominant form in many other tissues. SRF-S expression is restricted to vascular smooth muscle and SRF-I expression is restricted to the embryo. Transfection of SRF-L and SRF-M into C(2)C(12) cells showed that both forms are transactivators of the promoter of the smooth-muscle-specific gene **SM22alpha**, whereas SRF-I acted as a dominant negative form of SRF.

L75 ANSWER 5 OF 46 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 1999-326699 [27] WPIDS

AB WO 9921965 A UPAB: 19990714

NOVELTY - Neural crest cells (NCC) are stimulated to differentiate into vascular smooth muscle cells (VSMC) by culturing under conditions where expression of the SM22 alpha gene is induced.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method for identifying genes that regulate proliferation and migration of smooth muscle cells (SMC);

(2) a method for identifying agents (A) that modulate proliferation and/or migration of SMC; and

(3) modulating proliferation and/or migration of SMC using (A).

ACTIVITY - Antiproliferative; anti-atherosclerotic; antitumor.

MECHANISM OF ACTION - None given.

USE - This method of stimulating NCC differentiation is used to screen for agents (A) that modulate proliferation and/or migration of SMC.

(A) are particularly used:
(a) to treat or prevent unwanted proliferation of SMC, particularly in cases or restenosis or atherosclerosis; or
(b) to maintain an expanded lumenal volume after angioplasty or other vessel trauma (all claimed).
More generally they are useful for treating any vascular injury (e.g. caused by angioplasty, surgery etc.), coronary heart disease (e.g. myocardial ischemia, angina etc.), smooth muscle neoplasia (e.g. uterine fibroids or benign prostatic hypertrophy), obliterative diseases of vascular grafts or transplanted organs, etc.
The process can also be used in vitro, e.g. to produce SMC for subsequent use in vivo or in vitro.
ADVANTAGE - NCC can be rapidly and uniformly differentiated to SMC.
Dwg.0/17

L75 ANSWER 6 OF 46 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 2000-011873 [01] WPIDS
AB US 5949228 A UPAB: 20000524
NOVELTY - The feedback circuit includes a sensory circuit (110) that continually senses variations in process, temperature and power supply. The sensor information is provided to the top current mirror circuit (105) which adjusts its parameters accordingly.
USE - Feedback circuit for an integrated circuit.
ADVANTAGE - The feedback circuit improves the accuracy of the integrated circuit by compensating for process, temperature and power supply variations.
DESCRIPTION OF DRAWING(S) - The drawing shows a circuit diagram of the feedback circuit.
Top mirror circuit 105
Sensory circuit 110
Dwg.2/3

L75 ANSWER 7 OF 46 MEDLINE DUPLICATE 4
1999303750 Document Number: 99303750. Dominant negative murine serum response factor: alternative splicing within the activation domain inhibits transactivation of serum response factor binding targets.
Belaguli N S; Zhou W; Trinh T H; Majesky M W; Schwartz R J. (Departments of Cell Biology, Baylor College of Medicine, Houston, Texas 77030, USA.)
MOLECULAR AND CELLULAR BIOLOGY, (1999 Jul) 19 (7) 4582-91. Journal code: NGY. ISSN: 0270-7306. Pub. country: United States. Language: English.
AB Primary transcripts encoding the MADS box superfamily of proteins, such as MEF2 in animals and ZEMa in plants, are alternatively spliced, producing several isoformic species. We show here that murine serum response factor (SRF) primary RNA transcripts are alternatively spliced at the fifth exon, deleting approximately one-third of the C-terminal activation domain. Among the different muscle types examined, visceral smooth muscles have a very low ratio of SRFDelta5 to SRF. Increased levels of SRFDelta5 correlates well with reduced smooth muscle contractile gene activity within the elastic aortic arch, suggesting important biological roles for differential expression of SRFDelta5 variant relative to wild-type SRF. SRFDelta5 forms DNA binding-competent homodimers and heterodimers. SRFDelta5 acts as a naturally occurring dominant negative regulatory mutant that blocks SRF-dependent skeletal alpha-actin, cardiac alpha-actin, smooth alpha-actin, **SM22alpha**, and SRF promoter-luciferase reporter activities. Expression of SRFDelta5 interferes with differentiation of myogenic C2C12 cells and the appearance

of skeletal alpha-actin and myogenin mRNAs. SRFDelta5 repressed the serum-induced activity of the c-fos serum response element. SRFDelta5 fused to the yeast Gal4 DNA binding domain displayed low transcriptional activity, which was complemented by overexpression of the coactivator ATF6. These results indicate that the absence of exon 5 might be bypassed through recruitment of transcription factors that interact with extra-exon 5 regions in the transcriptional activating domain. The novel alternatively spliced isoform of SRF, SRFDelta5, may play an important regulatory role in modulating SRF-dependent gene expression.

L75 ANSWER 8 OF 46 MEDLINE DUPLICATE 5
2000040182 Document Number: 20040182. Medial localization of mineralization-regulating proteins in association with Monckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. Shanahan C M; Cary N R; Salisbury J R; Proudfoot D; Weissberg P L; Edmonds M E. (Department of Medicine, Addenbrooke's Hospital, Cambridge, UK.. cs131@mole.bio.cam.ac.uk) . CIRCULATION, (1999 Nov 23) 100 (21) 2168-76. Journal code: DAW. ISSN: 0009-7322. Pub. country: United States. Language: English.
AB BACKGROUND: Calcification of the media of peripheral arteries is referred to as Monckeberg's sclerosis (MS) and occurs commonly in aged and diabetic individuals. Its pathogenesis is unknown, but its presence predicts risk of cardiovascular events and leg amputation in diabetic patients. Several studies have documented expression of bone-associated genes in association with intimal atherosclerotic calcification, leading to the suggestion that vascular calcification may be a regulated process with similarities to developmental osteogenesis. Therefore, we examined gene expression in vessels with MS to determine whether there was evidence for a regulated calcification process in the vessel media. METHODS AND RESULTS: In situ hybridization, immunohistochemistry, and semiquantitative reverse-transcription polymerase chain reaction were used to examine the expression of mineralization-regulating proteins in human peripheral arteries with and without MS. MS occurred in direct apposition to medial vascular smooth muscle cells (VSMCs) in the absence of macrophages or lipid. These VSMCs expressed the smooth muscle-specific gene **SM22alpha** and high levels of matrix Gla protein but little osteopontin mRNA. Compared with normal vessels, vessels with MS globally expressed lower levels of matrix Gla protein and osteonectin, whereas alkaline phosphatase, bone sialoprotein, bone Gla protein, and collagen II, all indicators of osteogenesis/chondrogenesis, were upregulated. Furthermore, VSMCs derived from MS lesions exhibited osteoblastic properties and mineralized in vitro. CONCLUSIONS: These data indicate that medial calcification in MS lesions is an active process potentially orchestrated by phenotypically modified VSMCs.

L75 ANSWER 9 OF 46 MEDLINE DUPLICATE 6
1999225453 Document Number: 99225453. A role for serum response factor in coronary smooth muscle differentiation from proepicardial cells. Landerholm T E; Dong X R; Lu J; Belaguli N S; Schwartz R J; Majesky M W. (Departments of Pathology and Cell Biology and The Graduate Program in Cardiovascular Sciences, Baylor College of Medicine, Houston, TX 77030, USA.) DEVELOPMENT, (1999 May) 126 (10) 2053-62. Journal code: ECW. ISSN: 0950-1991. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Coronary artery smooth muscle (SM) cells originate from proepicardial cells that migrate over the surface of the heart, undergo epithelial to

mesenchymal transformation and invade the subepicardial and cardiac matrix. Prior to contact with the heart, proepicardial cells exhibit no expression of smooth muscle markers including SMalphaactin, **SM22alpha**, calponin, SMgammaactin or SM-myosin heavy chain detectable by RT-PCR or by immunostaining. To identify factors required for coronary smooth muscle differentiation, we excised proepicardial cells from Hamburger-Hamilton stage-17 quail embryos and examined them ex vivo. Proepicardial cells initially formed an epithelial colony that was uniformly positive for cytokeratin, an epicardial marker. Transcripts for flk-1, Nkx 2.5, GATA4 or smooth muscle markers were undetectable, indicating an absence of endothelial, myocardial or preformed smooth muscle cells. By 24 hours, cytokeratin-positive cells became SMalphaactin-positive. Moreover, serum response factor, undetectable in freshly isolated proepicardial cells, became strongly expressed in virtually all epicardial cells. By 72 hours, a subset of epicardial cells exhibited a rearrangement of cytoskeletal actin, focal adhesion formation and acquisition of a motile phenotype. Coordinately with mesenchymal transformation, calponin, **SM22alpha** and SMgammaactin became expressed. By 5-10 days, SM-myosin heavy chain mRNA was found, by which time nearly all cells had become mesenchymal. RT-PCR showed that large increases in serum response factor expression coincide with smooth muscle differentiation in vitro. Two different dominant-negative serum response factor constructs prevented the appearance of calponin-, **SM22alpha** - and SMgammaactin-positive cells. By contrast, dominant-negative serum response factor did not block mesenchymal transformation nor significantly reduce the number of cytokeratin-positive cells. These results indicate that the stepwise differentiation of coronary smooth muscle cells from proepicardial cells requires transcriptionally active serum response factor.

L75 ANSWER 10 OF 46 MEDLINE DUPLICATE 7

1999410804 Document Number: 99410804. Similarities and differences in smooth muscle alpha-actin induction by TGF-beta in smooth muscle versus non-smooth muscle cells. Hautmann M B; Adam P J; Owens G K. (Department of Molecular Physiology and Biological Physics, University of Virginia Health Sciences Center, Charlottesville 22908, USA.) ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, (1999 Sep) 19 (9) 2049-58. Journal code: B89. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB Transforming growth factor-beta (TGF-beta) has been shown to stimulate smooth muscle (SM) alpha-actin expression in smooth muscle cells (SMCs) and non-SMCs. We previously demonstrated that the 2 CArG boxes A and B and a novel TGF-beta control element (TCE) located within the first 125 bp of the SM alpha-actin promoter were required for TGF-beta inducibility of SM alpha-actin in SMCs. The aims of the present study were (1) to determine whether the TCE exhibits SMC specificity or contributes to TGF-beta induction of SM alpha-actin expression in non-SMCs (ie, endothelial cells and fibroblasts) and (2) to determine whether TGF-beta can induce expression of multiple TCE-containing SMC differentiation marker genes, such as **SM22alpha**, h(1) calponin, and SM myosin heavy chain (SM MHC) in non-SMCs. Results of transient transfection assays demonstrated that mutation of CArG A, CArG B, or the TCE within a 125-bp promoter context completely abolished TGF-beta inducibility of SM alpha-actin in endothelial cells and fibroblasts. However, in contrast to observations in

SMCs, inclusion of regions upstream from (-155) completely repressed TGF-beta responsiveness in non-SMCs. Electrophoretic mobility shift assays showed that TGF-beta enhanced binding of a serum response factor to the CArG elements and the binding of an as-yet-unidentified factor to the TCE in endothelial cells and fibroblasts, but to a much lesser extent compared with SMCs. TGF-beta also stimulated expression of the SMC differentiation marker **SM22alpha** in non-SMCs. However, in contrast to SMCs, TGF-beta did not induce expression of h(1) calponin and SM MHC in non-SMCs. In summary, these results suggest a conserved role for CArG A, CArG B, and the TCE in TGF-beta-induced expression of SM alpha-actin in SMCs and non-SMCs that is modified by a complex interplay of positive- and negative-acting cis elements in a cell-specific manner. Furthermore, observations that TGF-beta stimulated expression of several early but not late differentiation markers in non-SMCs indicate that TGF-beta alone is not sufficient to induce transdifferentiation of non-SMCs into SMCs.

L75 ANSWER 11 OF 46 MEDLINE DUPLICATE 8

2000104562 Document Number: 20104562. Vascular smooth muscle differentiation of murine stroma: a sequential model. Remy-Martin J P; Marandin A; Challier B; Bernard G; Deschaseaux M; Herve P; Wei Y; Tsuji T; Auerbach R; Dennis J E; Moore K A; Greenberger J S; Charbord P. (E.T.S. de Franche-Comte and Departement d'Information Medicale, Besancon, France.) EXPERIMENTAL HEMATOLOGY, (1999 Dec) 27 (12) 1782-95. Journal code: EPR. ISSN: 0301-472X. Pub. country: Netherlands. Language: English.

AB Previous studies by our group showed that stromal cells from human long-term marrow cultures were mesenchymal cells following a vascular smooth muscle pathway. The present study using 58 immortalized stromal lines from different hematopoietic sites was conducted to verify whether this hypothesis also held true for murine stroma. Principal components analysis performed using cytoskeletal and extracellular matrix proteins allowed the segregation of five factors explaining more than 70% of the variance. Factor I, including osteopontin and vimentin, and factor II, laminins and fibronectins, were representative of the mesenchyme. The remaining three factors were representative of vascular smooth muscle: factor III, including alphaSM actin, SM alpha actinin, **SM22alpha**, EDa+ fibronectin, and thrombospondin-1; factor IV, metavinculin and h-caldesmon; and factor V, smooth muscle myosin SM1 and desmin. All lines expressed factors I and II; 53 lines expressed factor III, 35 lines expressed factor IV; and 11 lines expressed factor V. A second principal components analysis including membrane antigens indicated the cosegregration of vascular cell adhesion molecule-1 with osteopontin and that of Ly6A/E with vimentin, whereas CD34 and Thy-1 appeared to be independent factors. The heterogeneity of vascular smooth muscle markers expression suggests that harmonious maintenance of hematopoiesis depends on the cooperation between different stromal cell clones.

L75 ANSWER 12 OF 46 MEDLINE DUPLICATE 9

1999293089 Document Number: 99293089. Smooth muscle-specific SM22 protein is expressed in the adventitial cells of balloon-injured rabbit carotid artery. Faggin E; Puato M; Zardo L; Franch R; Millino C; Sarinella F; Pauletto P; Sartore S; Chiavegato A. (Department of Experimental Medicine, University of Padua, Italy.) ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, (1999 Jun) 19 (6) 1393-404. Journal code: B89. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB During the "response-to-injury" process after a mechanical insult to the porcine coronary arteries, the adventitial cells acquire the structural characteristics of myofibroblasts before being incorporated into smooth muscle (SM) layer. We assessed whether the SM-specific SM22 protein can be used as a tracer of adventitial cell-myofibroblast differentiation in the mild balloon injury of rabbit carotid artery. To achieve this goal, we used 2 monoclonal anti-SM22 antibodies (E-11 and 1-B8) and a molecular probe for the **SM22alpha** mRNA isoform in immunocytochemical and in situ hybridization experiments. The differentiation profile and the migratory and proliferative ability of activated adventitial cells were evaluated by a panel of antibodies to some SM and nonmuscle antigens and pulse- and end-labeling with bromo-deoxyuridine, respectively. In adventitial cells, SM22 antigenicity and **SM22alpha** mRNA were detectable at days 2 and 4 and, to a lesser extent, at days 7 and 21 after injury, particularly near the adventitia-media interface and mostly colocalizing with bromo-deoxyuridine-positive cells. The pulse-labeling experiments showed that the large majority of these cells penetrated the outermost layer of the tunica media without migrating to the subendothelial region. The phenotypic features of activated migrating and nonmigrating adventitial cells resembled those of vimentin-actin myofibroblast subtype and fetal-type SM cells. These findings indicate that a direct exposure of adventitia to the lumen is not required for phenotypic changes and proliferation/migration of these cells. After comparison of the SM22 expression in arterial vessels during early stages of development, we hypothesize that in the injured carotid artery the mural incorporation of adventitial cells and the spatiotemporal activation of SM22 expression are reminiscent of the vascular morphogenetic process and suggest the existence of a stem cell-like reservoir in adventitia. The early adventitial upregulation of SM22 expression in the injured vessel might be related to a multistep transition process in which nonmuscle cells are converted to myofibroblasts and, possibly, to SM cells.

L75 ANSWER 13 OF 46 MEDLINE DUPLICATE 10
1999394341 Document Number: 99394341. Contribution of synthetic phenotype on the enhanced angiotensin II-generating system in vascular smooth muscle cells from spontaneously hypertensive rats. Fukuda N; Hu W Y; Satoh C; Nakayama M; Kishioka H; Kubo A; Kanmatsuse K. (Second Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan.) JOURNAL OF HYPERTENSION, (1999 Aug) 17 (8) 1099-107. Journal code: IEW. ISSN: 0263-6352. Pub. country: ENGLAND: United Kingdom. Language: English.

AB OBJECTIVE: We have demonstrated that cultured vascular smooth muscle cells (VSMC) from spontaneously hypertensive rats (SHR), but not from normotensive Wistar-Kyoto (WKY) rats, produce angiotensin II (Ang II) in a homogeneous culture with increased levels of angiotensinogen, cathepsin D and angiotensin converting enzyme (ACE) at early passages. In the current study, we investigated how changes in the cell phenotype affect the Ang II-generating system and the growth of VSMC from SHR. DESIGN AND METHODS: We evaluated basal DNA synthesis by [3H]thymidine incorporation, immunofluorescence of alpha-smooth muscle (SM) actin, mRNA expression of phenotype markers such as **SM22alpha** appeared by contractile phenotype, Ang II-generating system components and growth factors by reverse transcription and polymerase chain reaction analysis, and Ang II levels by radioimmunoassay in quiescent VSMC from WKY/Izumo rats and

SHR/Izumo at passages 4, 8 and 12. RESULTS: Basal DNA synthesis in VSMC from WKY rats increased with increasing passage number, whereas in cells from SHR it was markedly higher at early passages and was not affected by the passages. At early passage numbers, immunofluorescence of alpha-SM actin was stronger in VSMC from WKY rats than in cells from SHR, but decreased after several passages. Expression of **SM22alpha** mRNA was higher in VSMC from WKY rats than in cells from SHR at early passages, and decreased after several passages in cells from both rat strains. Expression of matrix Gla mRNA was higher in VSMC from SHR than in cells from WKY rats at early passage, and increased after several passages in cells from both rat strains. Ang II was not detected at early passages but increased in VSMC from WKY rats with increasing passage, whereas it was detected in VSMC from SHR at early passages and did not change with the passages. Expression of angiotensinogen mRNA was higher in VSMC from SHR than in cells from WKY rats, and was not affected by the passages. Expressions of cathepsin D and ACE mRNA were higher in VSMC from SHR than in cells from WKY rats at early passage, and were increased by the passages in VSMC from WKY rats. Expressions of transforming growth factor-beta1, platelet-derived growth factor A-chain, and basic fibroblast growth factor mRNA were significantly higher in VSMC from SHR than in cells from WKY rats, and were increased by the passages. CONCLUSION: These data indicate that early in culture VSMC from SHR have the synthetic phenotype, whereas VSMC from WKY rats have the contractile phenotype which then changes to the synthetic phenotype after increased passage numbers, with increased expression of cathepsin D and ACE, which produce Ang II, and increased expression of Ang II-related growth factors, which induce the exaggerated growth observed in VSMC from SHR.

L75 ANSWER 14 OF 46 MEDLINE DUPLICATE 11

2000029667 Document Number: 20029667. Human activin-A is expressed in the atherosclerotic lesion and promotes the contractile phenotype of smooth muscle cells. Engelse M A; Neele J M; van Achterberg T A; van Aken B E; van Schaik R H; Pannekoek H; de Vries C J. (Academic Medical Center, University of Amsterdam, Department of Biochemistry, Amsterdam and Erasmus University, Department of Endocrinology and Reproduction, Rotterdam, The Netherlands.) CIRCULATION RESEARCH, (1999 Nov 12) 85 (10) 931-9. Journal code: DAJ. ISSN: 0009-7330. Pub. country: United States. Language: English.

AB Activin is a member of the transforming growth factor-beta superfamily, and it modulates the proliferation and differentiation of various target cells. In this study, we investigated the role of activin in the initiation and progression of human atherosclerosis. The expression of activin, its physiological inhibitor follistatin, and activin receptors were assayed in human vascular tissue specimens that represented various stages of atherogenesis. In situ hybridization experiments revealed activin mRNA in endothelial cells and macrophages and a strong induction of activin expression in neointimal smooth muscle cells from the early onset of atherogenesis. We developed an "in situ free-activin binding assay" by using biotinylated follistatin, which allowed us to detect bioactive activin at specific sites in atherosclerotic lesions. The mRNAs encoding the activin receptors are expressed similarly in normal and atherosclerotic tissue, which indicates that activin-A signaling in atherogenesis is most likely dependent on changes in growth factor concentrations rather than on receptor levels. In vitro, activin induces

the contractile, nonproliferative phenotype in cultured smooth muscle cells, as is reflected by increased expression of smooth muscle-specific markers (SMalpha-actin and **SM22alpha**). Our data provide evidence that activin induces redifferentiation of neointimal smooth muscle cells, and we hypothesize that activin is involved in plaque stabilization.

L75 ANSWER 15 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS
1999:285967 Document No.: PREV199900285967. A transforming growth factor beta control element regulates **SM22alpha** gene expression in transgenic mice. Adam, P. J. (1); Regan, C. P. (1); Hautmann, M. B. (1); Owens, G. K. (1). (1) Dept. of Physiology, Univ. of Virginia, Charlottesville, VA, 22908 USA. FASEB Journal, (March 15, 1999) Vol. 13, No. 5 PART 2, pp. A694. Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology 99 Washington, D.C., USA April
17-21, 1999 Federation of American Societies for Experimental Biology. ISSN: 0892-6638. Language: English.

L75 ANSWER 16 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS
1999:169668 Document No.: PREV199900169668. Investigation of smooth muscle (SM) cell differentiation in vitro using **SM22alpha** promoter-reporter constructs. Ding, Rubai (1); Parmacek, Michael S.; D'Amore, Patricia (1). (1) Harvard Med. Sch., Schepens Eye Res. Inst., Boston, MA 02114 USA. FASEB Journal, (March 12, 1999) Vol. 13, No. 4 PART 1, pp. A531. Meeting Info.: Annual Meeting of the Professional Research Scientists for Experimental Biology 99 Washington, D.C., USA April 17-21, 1999 ISSN: 0892-6638. Language: English.

L75 ANSWER 17 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS
2000:24226 Document No.: PREV200000024226. **SM22alpha** promoter targets gene expression to vascular smooth muscle cells in vitro and in vivo. Akyurek, Levent M. (1); Yang, Zhiyong; Aoki, Kazunori; San, Hong; Nabel, Gary J.; Parmacek, Michael S.; Nabel, Elizabeth G.. (1) Univ of Michigan, Ann Arbor, MI USA. Circulation, (Nov. 2, 1999) Vol. 110, No. 18 SUPPL., pp. I.47. Meeting Info.: 72nd Scientific Sessions of the American Heart Association Atlanta, Georgia, USA November 7-10, 1999 ISSN: 0009-7322. Language: English.

L75 ANSWER 18 OF 46 CAPLUS COPYRIGHT 2000 ACS
1999:135807 Document No. 130:205885 Expression and regulation of a smooth muscle-specific gene, **sm22alpha**. Kim, Steven (Univ. of Chicago, Chicago, IL, USA). 190 pp. Avail. UMI, Order No. DA9841537 From: Diss. Abstr. Int., B 1999, 59(7), 3243 (English) 1998.
AB Unavailable

L75 ANSWER 19 OF 46 CAPLUS COPYRIGHT 2000 ACS
1998:239245 Document No. 128:304823 A smooth muscle-specific promoter of the
SM22.alpha. gene and its therapeutic uses. Parmacek, Michael S.; Solway, Julian (Arch Development Corporation, USA; Parmacek, Michael S.; Solway, Julian). PCT Int. Appl. WO 9815575 A1 19980416, 96 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US16204 19970829. PRIORITY: US 1996-726807 19961007.
AB A smooth muscle cell specific promoter from the SM22.alpha. gene of mouse is described for use in the smooth muscle-specific expression of

therapeutic genes. Genes expressed from this promoter can be used to prevent restenosis following balloon angioplasty and in methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, and in inhibition of contraction of smooth muscle. Characterization of the promoter region and the elements in it are reported,.

L75 ANSWER 20 OF 46 MEDLINE DUPLICATE 12
1998212009 Document Number: 98212009. Enhancement of serum-response factor-dependent transcription and DNA binding by the architectural transcription factor HMG-I(Y). Chin M T; Pellacani A; Wang H; Lin S S; Jain M K; Perrella M A; Lee M E. (Cardiovascular Biology Laboratory, Harvard School of Public Health, Boston, Massachusetts 02115, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Apr 17) 273 (16) 9755-60. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The mechanisms by which HMG-I proteins regulate cell growth are unknown, and their effects on gene expression have only been partially elucidated. We explored the potential interaction between HMG-I proteins and serum-response factor (SRF), a member of the MADS-box family of transcription factors. In cotransfection experiments, HMG-I(Y) potentiated SRF-dependent activation (by more than 5-fold) of two distinct SRF-responsive promoters, c-fos and the smooth muscle-specific gene **SM22alpha**. This effect was also observed with a heterologous promoter containing multiple copies of the CC(A/T)6GG (CArG) box. HMG-I proteins bound specifically to the CArG boxes of c-fos and **SM22alpha** in gel mobility shift analysis and enhanced binding of SRF to these CArG boxes. By chelating peptide-immobilized metal affinity chromatography, we mapped the domain of HMG-I(Y) that interacts with SRF to amino acids 50-81, a region that does not bind specifically to DNA in electrophoretic mobility shift assays even though it includes the third AT-hook DNA-binding domain. Surprisingly, HMG-I(Y) mutants that failed to bind DNA still enhanced SRF binding to DNA and SRF-dependent transcription. In contrast, deletion of the HMG-I(Y) 50-81 domain that bound SRF prevented enhancement of transcription. To our knowledge, this is the first report of an HMG-I protein interacting with a MADS-box transcription factor. Our observations suggest that members of the HMG-I family play an important role in SRF-dependent transcription and that their effect is mediated primarily by a protein-protein interaction.

L75 ANSWER 21 OF 46 MEDLINE DUPLICATE 13
1998234413 Document Number: 98234413. PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate [published erratum appears in J Cell Biol 1998 Jun 1;141(5):1287]. Hirschi K K; Rohovsky S A; D'Amore P A. (Harvard Medical School and Children's Hospital, Boston, Massachusetts 02115, USA.) JOURNAL OF CELL BIOLOGY, (1998 May 4) 141 (3) 805-14. Journal code: HMV. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB We aimed to determine if and how endothelial cells (EC) recruit precursors of smooth muscle cells and pericytes and induce their differentiation during vessel formation. Multipotent embryonic 10T1/2 cells were used as presumptive mural cell precursors. In an under-agarose coculture, EC induced migration of 10T1/2 cells via platelet-derived growth factor BB. 10T1/2 cells in coculture with EC changed from polygonal to spindle-shaped, reminiscent of smooth muscle cells in culture. Immunohistochemical and Western blot analyses were used to examine the expression of smooth muscle (SM)-specific markers in 10T1/2 cells cultured

in the absence and presence of EC. SM-myosin, **SM22alpha**, and calponin proteins were undetectable in 10T1/2 cells cultured alone; however, expression of all three SM-specific proteins was significantly induced in 10T1/2 cells cocultured with EC. Treatment of 10T1/2 cells with TGF-beta induced phenotypic changes and changes in SM markers similar to those seen in the cocultures. Neutralization of TGF-beta in the cocultures blocked expression of the SM markers and the shape change. To assess the ability of 10T1/2 cells to contribute to the developing vessel wall in vivo, prelabeled 10T1/2 cells were grown in a collagen matrix and implanted subcutaneously into mice. The fluorescently marked cells became incorporated into the medial layer of developing vessels where they expressed SM markers. These in vitro and in vivo observations shed light on the cell-cell interactions that occur during vessel development, as well as in pathologies in which developmental processes are recapitulated.

L75 ANSWER 22 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS
1999:72690 Document No.: PREV199900072690. Molecular control of vascular smooth muscle cell differentiation. Owens, G. K. (1). (1) Univ. Va. Sch. Med., Dep. Molecular Physiology Biol. Physics, Box 449, Room 2-29 Jordan Hall, Charlottesville, VA 22908 USA. Acta Physiologica Scandinavica, (Dec., 1998) Vol. 164, No. 4, pp. 623-635. ISSN: 0001-6772. Language: English.

AB Changes in the differentiated state of the vascular smooth muscle cell (SMC) including enhanced growth responsiveness, altered lipid metabolism, and increased matrix production are known to play a key role in development of atherosclerotic disease. As such, there has been extensive interest in understanding the molecular mechanisms and factors that regulate differentiation of vascular SMC, and how this regulation might be disrupted in vascular disease. Key questions include determination of mechanisms that control the coordinate expression of genes required for the differentiated function of the smooth muscle cell, and determination as to how these regulatory processes are influenced by local environmental cues known to be important in control of smooth muscle differentiation. Of particular interest, a number of common cis regulatory elements including highly conserved CArG (CC(A/T)6GG) motifs or CArG-like motifs and a TGFbeta control element have been identified in the promoters of virtually all smooth muscle differentiation marker genes characterized to date including smooth muscle alpha-actin, smooth muscle myosin heavy chain, telokin, and **SM22alpha** and shown to be required for expression of these genes both in vivo and in vitro. In addition, studies have identified a number of trans factors that interact with these cis elements, and shown how the expression or activity of these factors is modified by local environmental cues such as contractile agonists that are known to influence differentiation of smooth muscle.

L75 ANSWER 23 OF 46 MEDLINE DUPLICATE 14
1998173341 Document Number: 98173341. Calcification of human vascular cells in vitro is correlated with high levels of matrix Gla protein and low levels of osteopontin expression. Proudfoot D; Skepper J N; Shanahan C M; Weissberg P L. (Department of Medicine, University of Cambridge, Addenbrooke's Hospital, England.. dp@mole.bio.cam.ac.uk) . ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, (1998 Mar) 18 (3) 379-88. Journal code: B89. ISSN: 1079-5642. Pub. country: United States.

Language: English.

AB The cellular and molecular events leading to calcification in atherosclerotic lesions are unknown. We and others have shown that bone-associated proteins, particularly matrix Gla protein (MGP) and osteopontin (OP), can be detected in atherosclerotic lesions, thus suggesting an active calcification process. In the present study, we aimed to determine whether human vascular smooth muscle cells (VSMCs) could calcify in vitro and to determine whether MGP and OP have a role in vascular calcification. We established that human aortic VSMCs and placental microvascular pericytes spontaneously form nodules in cell culture and induce calcification, as detected by von Kossa's method, Alizarin red S staining, and electron microscopy. The cells in calcifying nodules differed from those in monolayer cultures by expressing higher levels of the SMC markers alpha-SM actin, **SM22alpha**, and calponin. In addition, Northern blot analysis revealed that in human VSMCs, calcification was associated with increased levels of MGP mRNA. In contrast, OP mRNA was barely detectable in calcified human VSMCs and pericyte nodules, nor was OP protein detected, suggesting that OP was not necessary for calcification to occur. These studies reveal that human VSMCs are capable of inducing calcification and that MGP may have a role in human vascular calcification.

L75 ANSWER 24 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS

1999:511545 Document No.: PREV199900511545. Spatial and temporal expression of smooth muscle-specific protein, **SM22alpha**, during Xenopus laevis embryogenesis. Oka, Toru; Monzen, Koshiro; Kudoh, Sumiyo; Hiroi, Yukio; Shiojima, Ichiro. Univ. Tokyo Sch. Med., Tokyo Japan. Circulation, (Oct. 27, 1998) Vol. 98, No. 17 SUPPL., pp. I333. Meeting Info.: 71st Scientific Sessions of the American Heart Association Dallas, Texas, USA November 8-11, 1998 The American Heart Association. ISSN: 0009-7322. Language: English.

L75 ANSWER 25 OF 46 MEDLINE DUPLICATE 15

1998307252 Document Number: 98307252. Expression of the smooth muscle calponin gene in human osteosarcoma and its possible association with prognosis. Yamamura H; Yoshikawa H; Tatsuta M; Akedo H; Takahashi K. (Department of Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan.) INTERNATIONAL JOURNAL OF CANCER, (1998 Jun 19) 79 (3) 245-50. Journal code: GQU. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB The basic calponin gene is a smooth muscle differentiation-specific gene that encodes an actin-binding protein involved in the regulation of smooth muscle contractility. We studied the expression of the calponin gene in 8 human osteosarcoma cell lines and 17 primary human osteosarcoma tissues by RT-PCR analysis. We also analyzed mRNA expression of smooth muscle-specific genes including **SM22alpha**, caldesmon and alpha-actin, and for neutral and acidic calponin isoforms. The genes were expressed at various levels by osteosarcoma cell lines and tissues of diverse histological subtypes. The basic calponin protein of an expected size was detected in osteosarcoma cell lines by immunoblot analysis and was localized by immunohistochemistry in the cytoplasm of the tumor cells in osteosarcoma tissues. Survival was found to be significantly increased in patients whose tumors exhibited basic calponin expression, compared with those with no expression. Alterations in the expression of other markers examined were not correlated with prognosis. Our results suggest that the basic calponin gene product may be a novel prognostic variable in

patients with osteosarcoma.

L75 ANSWER 26 OF 46 MEDLINE DUPLICATE 16
1998139204 Document Number: 98139204. Expression of PDGF alpha-receptor in renal arteriosclerosis and rejecting renal transplants. Floege J; Hudkins K L; Davis C L; Schwartz S M; Alpers C E. (Division of Nephrology, Medizinische Hochschule, Hannover, Germany.) JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY, (1998 Feb) 9 (2) 211-23. Journal code: A6H. ISSN: 1046-6673. Pub. country: United States. Language: English.
AB Platelet-derived growth factor (PDGF) plays an important role in renal disease. We have recently demonstrated that in healthy mature human kidney, PDGF alpha-receptor expression is largely restricted to interstitial cells. The study presented here assesses the expression of PDGF alpha-receptor in 18 mature adult kidneys with arteriosclerosis from individuals with no clinically evident history of renal disease other than localized neoplasia, in 13 kidneys with irreversible transplant rejection, and in a series of renal transplant biopsies composed of examples of both severe and absent rejection, by in situ hybridization and immunocytochemistry. Strong focal or diffuse expression of PDGF alpha-receptor mRNA and protein was noted in some intimal cells of intrarenal arterial vessels exhibiting signs of arteriosclerosis and/or vascular rejection. By double immunostaining, it could be shown that these cells were neither endothelial cells nor infiltrating leukocytes. The cells were most often identified as smooth muscle by colabeling for the smooth muscle cell-specific protein **SM22alpha** and less commonly for alpha-smooth muscle actin. There was also a population of PDGF alpha-receptor-expressing cells that failed to colabel with any of these markers, and hence remain of uncertain histogenesis. These intimal cells were generally negative for several other markers of differentiated smooth muscle cells, i.e., calponin and desmin. Near these PDGF alpha-receptor-positive intimal cells, expression of PDGF A-chain, an alpha-receptor ligand, was demonstrated in endothelial, intimal, and/or medial cells. Prominent PDGF alpha-receptor mRNA and protein expression also was noted in areas of interstitial fibrosis and in some glomeruli, in particular those with segmental glomerulosclerosis or fibrotic crescents. Double immunolabeling for PDGF alpha-receptor and alpha-smooth muscle actin confirmed that most of these latter PDGF alpha-receptor-positive cells were interstitial myofibroblasts or mesangial cells, or both. In summary, these data demonstrate widespread expression of PDGF alpha-receptor in renal cell types involved in fibrotic and sclerosing processes. The data also show that PDGF alpha-receptor expression identifies a unique population of phenotypically altered vascular smooth muscle cells, which appear to be involved in the vascular response to injury.

L75 ANSWER 27 OF 46 MEDLINE DUPLICATE 17
1998360104 Document Number: 98360104. Paralogous **sm22alpha** (Tagln) genes map to mouse chromosomes 1 and 9: further evidence for a paralogous relationship. Stanier P; Abu-Hayyeh S; Murdoch J N; Eddleston J; Copp A J. (Division of Paediatrics, Obstetrics and Gynaecology, Queen Charlotte's and Chelsea Hospital, Goldhawk Road, London, W6 OXG, United Kingdom.. pstanier@rpms.ac.uk) . GENOMICS, (1998 Jul 1) 51 (1) 144-7. Journal code: GEN. ISSN: 0888-7543. Pub. country: United States. Language: English.
AB **SM22alpha** (TAGLN) is one of the earliest markers of

differentiated smooth muscle, being expressed exclusively in the smooth muscle cells of adult tissues and transiently in embryonic skeletal and cardiac tissues. We have identified and mapped the mouse Tagln gene and a closely related gene, **Sm22alpha** homolog (Tagln2). The chromosomal localization for Tagln was identified by linkage analysis to distal mouse chromosome 9 between D9Mit154 and D9Mit330, closely linked to the anchor locus D9Nds10. The localization of Tagln2 was also determined and was found to map between Fcgr2 and D1Mit149 on distal mouse chromosome 1. This localization is homologous to a region of human 1q21-q25 to which an EST representing human TAGLN2 was previously mapped. The two regions, distal mouse chromosome 1 and proximal mouse chromosome 9, and the human regions with conserved synteny (1q21-q25 and 11q22-qter) are believed to be paralogous, reflecting either conserved remnants of duplicated chromosomes or segments of chromosomes during vertebrate evolution.

L75 ANSWER 28 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS
1998:294674 Document No.: PREV199800294674. Expression of **SM22alpha** in human prostate. Lin, Victor K.; Boetticher, Nicholas C.; Lemack, Gary E.; Word, R. Ann; McConnell, John D.. Dallas, TX USA. Journal of Urology, (May, 1998) Vol. 159, No. 5 SUPPL., pp. 108. Meeting Info.: 93rd Annual Meeting of the American Urological Association, Inc. San Diego, California, USA May 30-June 4, 1998 American Urological Association. ISSN: 0022-5347. Language: English.

L75 ANSWER 29 OF 46 MEDLINE DUPLICATE 18
1998315127 Document Number: 98315127. Role of Endothelin-1/Endothelin-A receptor-mediated signaling pathway in the aortic arch patterning in mice [see comments]. Yanagisawa H; Hammer R E; Richardson J A; Williams S C; Clouthier D E; Yanagisawa M. (Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-9050, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1998 Jul 1) 102 (1) 22-33. Journal code: HS7. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB The intercellular signaling mediated by endothelins and their G protein-coupled receptors has recently been shown to be essential for the normal embryonic development of subsets of neural crest cell derivatives. Endothelin-1 (ET-1) is proteolytically generated from its inactive precursor by endothelin-converting enzyme-1 (ECE-1) and acts on the endothelin-A (ETA) receptor. Genetic disruption of this ET-1/ECE-1/ETA pathway results in defects in branchial arch- derived craniofacial tissues, as well as defects in cardiac outflow and great vessel structures, which are derived from cephalic (cardiac) neural crest. In this study, in situ hybridization of ETA-/- and ECE-1(-)/- embryos with a cardiac neural crest marker, cellular retinoic acid-binding protein-1, shows that the migration of neural crest cells from the neural tube to cardiac outflow tract is not affected in these embryos. Immunostaining of an endothelial marker, platelet endothelial cell adhesion molecule CD-31, shows that the initial formation of the branchial arch arteries is not disturbed in ETA-/- or ECE-1(-)/- embryos. To visualize the subsequent patterning of arch vessels in detail, we generated ETA-/- or ECE-1(-)/- embryos that expressed an **SM22alpha**-lacZ marker transgene in arterial smooth muscle cells. Wholemount X-gal staining of these mutant embryos reveals that the abnormal regression and persistence of specific arch arteries results in disturbance of asymmetrical remodeling of the arch arteries. These defects include abnormal regression of arch arteries 4 and 6, enlargement of arch artery 3, and abnormal persistence of the

bilateral ductus caroticus and right dorsal aorta. These abnormalities eventually lead to various types of great vessel malformations highly similar to those seen in neural crest-ablated chick embryos and human congenital cardiac defects. This study demonstrates that ET-1/ETA-mediated signaling plays an essential role in a complex process of aortic arch patterning by affecting the postmigratory cardiac neural crest cell development.

L75 ANSWER 30 OF 46 MEDLINE DUPLICATE 19

97220019 Document Number: 97220019. A serum response factor-dependent transcriptional regulatory program identifies distinct smooth muscle cell sublineages. Kim S; Ip H S; Lu M M; Clendenin C; Parmacek M S. (Department of Medicine, University of Chicago, Illinois 60637, USA.) MOLECULAR AND CELLULAR BIOLOGY, (1997 Apr) 17 (4) 2266-78. Journal code: NGY. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB The **SM22alpha** promoter has been used as a model system to define the molecular mechanisms that regulate smooth muscle cell (SMC) specific gene expression during mammalian development. The **SM22alpha** gene is expressed exclusively in vascular and visceral SMCs during postnatal development and is transiently expressed in the heart and somites during embryogenesis. Analysis of the **SM22alpha** promoter in transgenic mice revealed that 280 bp of 5' flanking sequence is sufficient to restrict expression of the lacZ reporter gene to arterial SMCs and the myotomal component of the somites. DNase I footprint and electrophoretic mobility shift analyses revealed that the **SM22alpha** promoter contains six nuclear protein binding sites (designated smooth muscle elements [SMEs] -1 to -6, respectively), two of which bind serum response factor (SRF) (SME-1 and SME-4). Mutational analyses demonstrated that a two-nucleotide substitution that selectively eliminates SRF binding to SME-4 decreases **SM22alpha** promoter activity in arterial SMCs by approximately 90%. Moreover, mutations that abolish binding of SRF to SME-1 and SME-4 or mutations that eliminate each SME-3 binding activity totally abolished **SM22alpha** promoter activity in the arterial SMCs and somites of transgenic mice. Finally, we have shown that a multimerized copy of SME-4 (bp -190 to -110) when linked to the minimal **SM22alpha** promoter (bp -90 to +41) is necessary and sufficient to direct high-level transcription in an SMC lineage-restricted fashion. Taken together, these data demonstrate that distinct transcriptional regulatory programs control **SM22alpha** gene expression in arterial versus visceral SMCs. Moreover, these data are consistent with a model in which combinatorial interactions between SRF and other transcription factors that bind to SME-4 (and that bind directly to SRF) activate transcription of the **SM22alpha** gene in arterial SMCs.

L75 ANSWER 31 OF 46 MEDLINE

97426462 Document Number: 97426462. Transcriptional targeting of replication-defective adenovirus transgene expression to smooth muscle cells in vivo. Kim S; Lin H; Barr E; Chu L; Leiden J M; Parmacek M S. (Department of Medicine, The University of Chicago, Chicago, Illinois 60637, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1997 Sep 1) 100 (5) 1006-14. Journal code: HS7. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Gene transfer using replication-defective adenoviruses (RDAd) holds promise for the treatment of vascular proliferative disorders, but is potentially limited by the capacity of these viruses to infect multiple cell lineages. We have generated an RDAd vector, designated AdSM22-lacZ, which encodes the bacterial lacZ reporter gene under the transcriptional control of the smooth muscle cell (SMC)-specific **SM22alpha**

promoter. Here, we show that in vitro AdSM22-lacZ programs expression of the lacZ reporter gene in primary rat aortic SMCs and immortalized A7r5 SMCs, but not in primary human umbilical vein endothelial cells (HUVECs) or NIH 3T3 cells. Consistent with these results, after intraarterial administration of AdSM22-lacZ to control and balloon-injured rat carotid arteries, beta-galactosidase activity was detected within SMCs of the tunica media and neointima, but not within endothelial or adventitial cells. Moreover, intravenous administration of AdSM22-lacZ did not result in lacZ gene expression in the liver or lungs. Finally, we have shown that direct injection of AdSM22-lacZ into SMC-containing tissues such as the ureter and bladder results in high-level transgene expression in visceral SMCs. Taken together, these results demonstrate that transgene expression after infection with an RDAd vector can be regulated in an SMC lineage-restricted fashion by using a transcriptional cassette containing the SMC-specific **SM22alpha** promoter. The demonstration of an efficient gene delivery system targeted specifically to SMCs provides a novel means to restrict expression of recombinant gene products to vascular or visceral SMCs in vivo.

L75 ANSWER 32 OF 46 MEDLINE DUPLICATE 20

97384931 Document Number: 97384931. Evidence for serum response factor-mediated regulatory networks governing **SM22alpha** transcription in smooth, skeletal, and cardiac muscle cells. Li L; Liu Z; Mercer B; Overbeek P; Olson E N. (Department of Internal Medicine, Wayne State University, Detroit, Michigan 48335, USA.) DEVELOPMENTAL BIOLOGY, (1997 Jul 15) 187 (2) 311-21. Journal code: E7T. ISSN: 0012-1606. Pub. country: United States. Language: English.

AB **SM22alpha** is an adult smooth muscle-specific protein that is expressed in the smooth, cardiac, and skeletal muscle lineages during early embryogenesis before becoming restricted specifically to all vascular and visceral smooth muscle cells (SMC) in late fetal development and adulthood. We have used the **SM22alpha** gene as a marker to define the regulatory mechanisms that control muscle-specific gene expression in SMCs. Previously, we reported that the 445-base-pair promoter of **SM22alpha** was sufficient to direct transcription of a lacZ reporter gene in early cardiac and skeletal muscle cell lineages and in a subset of arterial SMCs, but not in venous nor visceral SMCs in transgenic mice. Here we describe two evolutionarily conserved CArG (CC(A/T)6GG) boxes in the **SM22alpha** promoter, both of which are essential for full promoter activity in cultured SMCs. In contrast, only the promoter-proximal CArG box is essential for specific expression in developing smooth, skeletal, and cardiac muscle lineages in transgenic mice. Both CArG boxes bind serum response factor (SRF), but SRF binding is not sufficient for **SM22alpha** promoter activity, since overexpression of SRF in the embryonal teratocarcinoma cell line F9, which normally expresses low levels of SRF, fails to activate the promoter. However, a chimeric protein in which SRF was fused to the transcription activation domain of the viral coactivator VP16 is able to activate the **SM22alpha** promoter in F9 cells. These results demonstrate the **SM22alpha** promoter-proximal CArG box is a target for the regulatory programs that confer smooth, skeletal, and cardiac muscle specificity to the **SM22alpha** promoter and they suggest that SRF activates **SM22alpha** transcription in conjunction with additional regulatory factors that are cell type-restricted.

L75 ANSWER 33 OF 46 MEDLINE DUPLICATE 21

1998043848 Document Number: 98043848. Fibroblast transgelin and smooth muscle **SM22alpha** are the same protein, the expression of which

is down-regulated in many cell lines. Lawson D; Harrison M; Shapland C. (Department of Molecular Pathology, UCL Medical School, Windeyer Institute of Medical Sciences, London, United Kingdom.) CELL MOTILITY AND THE CYTOSKELETON, (1997) 38 (3) 250-7. Journal code: CRD. ISSN: 0886-1544. Pub. country: United States. Language: English.

AB In this report we investigate the expression and relationship of transgelin (Tg), a transformation and shape-change sensitive actin gelling protein found in fibroblasts and smooth muscle [Shapland et al., 1988: J. Cell. Biol. 107:153-161; Shapland et al., 1993: J. Cell. Biol. 121:1065-1073], to **SM22alpha**, a smooth muscle protein of unknown function [Lees-Millar et al., 1987: J. Biol. Chem. 262:2988-2993; Solway et al., 1995: J. Biol. Chem. 270:13460-13469]. To clarify their relationship we have cloned and sequenced the cDNA encoding Tg from cultures of rat embryo fibroblasts. The sequences of fibroblast Tg and the smooth muscle isoform SM22 are identical [Prinjha et al., 1994: Cell Motil. Cytoskeleton 28:243-255; Shanahan et al., 1993: Circ. Res. 73:193-204; Solway et al., 1995]. These data, coupled with our immunoblot analysis and previous observations [Shapland et al., 1988; Shapland et al., 1993], demonstrate that Tg expression is not restricted to smooth muscle since this protein is also present in normal mesenchymal cells. However, we also show that Tg, although present in secondary cultures of mouse and rat embryo fibroblasts, is absent in many apparently normal fibroblast cell lines. Tg down-regulation may therefore be an early and sensitive marker for the onset of transformation. A functional role for Tg is unlikely to directly involve Ca2+ since it neither contains a functional EF hand nor binds 45Ca2+.

L75 ANSWER 34 OF 46 MEDLINE DUPLICATE 22

97420698 Document Number: 97420698. Structure and expression of the human **SM22alpha** gene, assignment of the gene to chromosome 11, and repression of the promoter activity by cytosine DNA methylation. Yamamura H; Masuda H; Ikeda W; Tokuyama T; Takagi M; Shibata N; Tatsuta M; Takahashi K. (Department of Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases.) JOURNAL OF BIOCHEMISTRY, (1997 Jul) 122 (1) 157-67. Journal code: HIF. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB To investigate the molecular mechanisms that control expression of smooth muscle cell (SMC) differentiation genes, we have isolated the human SM22a gene, which is composed of five exons and four introns, spanning an approximately 6-kilobase (kb) genomic DNA at chromosome region 11q23.2. Expression of the SM22a messenger RNA was detected in serum-stimulated cell cultures including SMC, undifferentiated skeletal muscle-lineage cells, and fibroblasts, and it was down-regulated in SMC of balloon-injured atheromatous human vessels. A major transcription start site of the **SM22alpha** gene is located at 75 base-pairs (bp) upstream of the ATG start codon. Analysis of the 2.6 kb 5'-upstream sequence demonstrated that two CArG/SRF-boxes and two GC-box/Sp1-binding sites were present at bp -147 and -274, and at bp -233 and -1635, respectively. The nucleotide sequences of the two CArG/SRF-boxes and the proximal GC-box/Sp1 binding site are 100% conserved with those of the murine **SM22alpha** genes [Solway, J., Seltzer, J., Samaha, F.F., Kim, S., Alger, L.E., Niu, Q., Morriesey, E.E., Ip, H.S., and Parmacek, M.S. (1995) J. Biol. Chem. 270, 13460-13469; Kemp, P.R., Osbourn, J.K., Grainger, D.J., and Metcalf, C. (1995) Biochem. J. 310, 1037-1043]. Cell transfection assays using a luciferase reporter gene construct containing the 455-bp 5'-flanking region (positions -26 to -480) showed that methylation of the CpG dinucleotides within this segment reduces its

transcriptional activity. The results imply a novel mechanism for transcriptional control of the SMC differentiation-specific gene promoter.

L75 ANSWER 35 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS
1998:2481 Document No.: PREV199800002481. The A10 cell line: A model for neonatal, neointimal, or differentiated vascular smooth muscle cells. Rao, Rohini S.; Miano, Joseph M.; Olson, Eric N.; Seidel, Charles L. (1). (1) Dep. Med., Sect. Cardiovasc. Sci., Room 512C, Baylor Coll. Med., One Baylor Plaza, Houston, TX 77030 USA. Cardiovascular Research, (Oct., 1997) Vol. 36, No. 1, pp. 118-126. ISSN: 0008-6363. Language: English.
AB Objectives: The A10 cell line was derived from the thoracic aorta of embryonic rat and is a commonly used model of vascular smooth muscle cells (VSMC). Despite its wide use this cell line has not been well characterized. This is especially important in light of recent evidence of phenotypically distinct cell populations isolated from rat vascular tissue. Therefore, the present study was undertaken to confirm the VSMC nature of A10 cells and to investigate whether these cells particularly resemble adult, neonatal, or neointimal rat VSMC. Methods: A variety of defining characteristics were used that included immunofluorescent analysis for smooth muscle alpha-actin, smooth and non-muscle myosin heavy chains, desmin and vimentin; Western analysis for smooth muscle and non-muscle myosin heavy chains; mRNA analysis for smooth muscle myosin heavy chain, calponin, **SM22alpha**, tropoelastin and PDGF-B peptide; and function assays of cell migration, proliferation and agonist induced intracellular Ca transients. Results: A10 cells expressed smooth muscle alpha-actin, **SM22alpha**, smooth muscle calponin and vimentin, characteristic of in vivo rat VSMCs; however they also resembled de-differentiated smooth muscle cells in that they expressed non-muscle myosin rather than smooth muscle myosin heavy chain. A10 cells resembled cultured rat neonatal smooth muscle cells ("pup cells") in that they had an epithelioid shape and lacked functional PDGF-alpha receptors; however they did not express PDGF-B mRNA or proliferate in low serum containing medium as do neonatal cells. A10 cells had several characteristics in common with neointimal cells including the expression of alpha-actin, vimentin, and non-muscle myosin and the lack of expression of PDGF-B mRNA as well as the ability to migrate in response to PDGF-BB. Conclusion: In conclusion, A10 cells are nondifferentiated VSMC that differ from neonatal but bear significant resemblance to neointimal cells.

L75 ANSWER 36 OF 46 MEDLINE
96180279 Document Number: 96180279. Expression of the **SM22alpha** promoter in transgenic mice provides evidence for distinct transcriptional regulatory programs in vascular and visceral smooth muscle cells. Li L; Miano J M; Mercer B; Olson E N. (Department of Biochemistry and Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, 77030, USA.) JOURNAL OF CELL BIOLOGY, (1996 Mar) 132 (5) 849-59. Journal code: HMV. ISSN: 0021-9525. Pub. country: United States. Language: English.
AB **SM22alpha** is a putative calcium-binding protein that is expressed in cardiac, smooth, and skeletal muscle lineages during mouse embryogenesis and in adult smooth muscle cells (SMC). To define the

mechanisms that regulate smooth muscle-specific gene transcription, we isolated the **SM22alpha** gene and analyzed its 5'-flanking region for elements that direct smooth muscle expression in transgenic mice. Using a series of promoter deletions, a region of the **SM22alpha** promoter containing 445 base pairs of 5'-flanking sequence was found to be sufficient to direct the specific expression of a lacZ transgene in mouse embryos in the vascular smooth, cardiac, and skeletal muscle lineages in a temporospatial pattern similar to the endogenous **SM22alpha** gene. However, in contrast to the endogenous gene, transgene expression was not detected in venous, nor visceral SMCs. This **SM22alpha**-lacZ transgene was therefore able to distinguish between the transcriptional regulatory programs that control gene expression in vascular and visceral SMCs and revealed heretofore unrecognized differences between these SMC types. These results suggest that distinct transcriptional regulation programs control muscle gene expression in vascular and visceral SMCs.

L75 ANSWER 37 OF 46 CAPLUS COPYRIGHT 2000 ACS
1996:729973 Document No. 126:57812 Presence of Ca2+-sensitive and -insensitive SM22.alpha. isoproteins in bovine aorta. Shishibori, Tsuyoshi; Yamashita, Kayoko; Bandoh, Junko; Oyama, Yuhta; Kobayashi, Ryoji (School Medicine, Kagawa medical University, Miki, 761-07, Japan). Biochem. Biophys. Res. Commun., 229(1), 225-230 (English) 1996. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Academic.
AB Two proteins (25- and 22-kDa) that cross-react with anti-gizzard SM22.alpha. antibody were isolated from bovine aorta. The former, identical to a SM22.alpha. homolog reported previously (Kobayashi, R., Kubota, T., and Hidaka, H. (1994) Biochem. Biophys. Res. Commun. 198, 1275-1280), assocs. with the membrane fraction in the presence of Ca2+ and dissocs. in the presence of EGTA, while the latter is insensitive to Ca2+. Peptide mapping and partial sequence anal. revealed that the 22-kDa protein is an isoform of the 25-kDa protein, without 22 amino acid residues from the C-terminus. Immunol. anal. of the tissue distribution of these proteins showed that the 25-kDa protein exists in lung and spleen besides aorta, while the 22-kDa protein occurs specifically in the aorta and spleen.

L75 ANSWER 38 OF 46 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 23
1996:75819 Document No. 124:171478 SM22.alpha., a marker of adult smooth muscle, is expressed in multiple myogenic lineages during embryogenesis. Li, Li; Miano, Joseph M.; Cserjesi, Peter; Olson, Eric N. (M. D. Anderson Cancer Center, Univ. of Texas, Houston, TX, 75235-9148, USA). Circ. Res., 78(2), 188-95 (English) 1996. CODEN: CIRUAL. ISSN: 0009-7330.
AB SM22.alpha. is a calponin-related protein that is expressed specifically in adult smooth muscle. To begin to define the mechanisms that regulate the establishment of the smooth muscle lineage, the expression pattern of the SM22.alpha. gene was analyzed during mouse embryogenesis. In situ hybridization demonstrated that SM22.alpha. transcripts were first expressed in vascular smooth muscle cells at about embryonic day (E) 9.5 and thereafter continued to be expressed in all smooth muscle cells into adulthood. In contrast to its smooth muscle specificity in adult tissue, SM22.alpha. was expressed transiently in the heart between E8.0 and E12.5 and in skeletal muscle cells in the myotomal compartment of the somites between E9.5 and E12.5. The expression of SM22.alpha. in smooth muscle cells, as well as early cardiac and skeletal muscle cells, suggests that

there may be commonalities between the regulatory programs that direct muscle-specific gene expression in these 3 myogenic cell types.

L75 ANSWER 39 OF 46 BIOSIS COPYRIGHT 2000 BIOSIS
1997:3015 Document No.: PREV199799302218. Functional characterization of the vascular smooth muscle cell-specific **SM22alpha** promoter in transgenic mice. Kim, Steven; Parmacek, Michael S.. Univ. Chicago, Chicago, IL USA. Circulation, (1996) Vol. 94, No. 8 SUPPL., pp. I166. Meeting Info.: 69th Scientific Sessions of the American Heart Association New Orleans, Louisiana, USA November 10-13, 1996 ISSN: 0009-7322. Language: English.

L75 ANSWER 40 OF 46 CAPLUS COPYRIGHT 2000 ACS
1995:609216 Document No. 123:190188 Structure and expression of a smooth muscle cell-specific gene, SM22.alpha.. Solway, Julian; Seltzer, Jonathan; Samaha, Frederick F.; Kim, Steven; Alger, Linda E.; Niu, Qun; Morrisey, Edward E.; Ip, Hon S.; Parmacek, Michael S. (Dep. Med., Univ. Chicago, Chicago, IL, 60637, USA). J. Biol. Chem., 270(22), 13460-9 (English) 1995. CODEN: JBCHA3. ISSN: 0021-9258.
AB SM22.alpha. is expressed exclusively in smooth muscle-contg. tissues of adult animals and is one of the earliest markers of differentiated smooth muscle cells (SMCs). To examine the mol. mechanisms that regulate SMC-specific gene expression, the murine Sm22.alpha. gene was isolated and structurally characterized. SM22.alpha. is a 6.2-kb single copy gene composed of 5 exons. SM22.alpha. mRNA is expressed at high levels in the aorta, uterus, lung, and intestine, and in primary cultures of rat aortic SMCs, and the SMC line, A7r5. In contrast to genes encoding SMC contractile proteins, SM22.alpha. gene expression is not decreased in proliferating SMCs. Transient transfection expts. demonstrated that 441 bp of SM22.alpha. 5'-flanking sequence was necessary and sufficient to program high level transcription of a luciferase reporter gene in both primary rat aortic SMCs and A7r5 cells. DNA sequence analyses revealed that the 441-bp promoter contains two CArG/SRF boxes, a CACC box, and one potential MEF-2 binding site, cis-acting elements which are each important regulators of striated muscle transcription. Taken together, these studies have identified the murine SM22.alpha. promoter as an excellent model system for studies of developmentally regulated, lineage-specific gene expression in SMCs.

L75 ANSWER 41 OF 46 CAPLUS COPYRIGHT 2000 ACS
1995:822790 Document No. 123:331655 Cloning and analysis of the promoter region of the rat SM22.alpha. gene. Kemp, Paul R.; Osbourn, Jane K.; Grainger, David J.; Metcalfe, James C. (Dep. Biochem., Cambridge Univ., Cambridge, CB2 1QW, UK). Biochem. J., 310(3), 1037-43 (English) 1995. CODEN: BIJOAK. ISSN: 0264-6021.
AB The authors have cloned and sequenced a 1.9-kb fragment of the 5'-upstream sequence of the smooth-muscle-specific gene SM22.alpha.. The region cloned consisted of the SM22.alpha. promoter, a 65-bp exon contg. most of the 5'-untranslated region and 307 bp of the first intron. A 1.5-kb fragment at the 5' end of this sequence was able to drive the expression of a reporter chloramphenicol acetyltransferase (CAT) gene in both vascular smooth-muscle cells and Rat-1 fibroblasts. This promoter region did not contain a consensus TATAA box but contained the sequence TTTAAA 25 bp from the major start site identified by primer extension. Deletion anal. showed that a fragment of the promoter from +65 to -303 was more active in both cell types than the 1.5-kb fragment suggesting that there are silencer sequences in the region 5' to the core promoter. CAT

activity was also obsd. with fragments contg. bases +65 to -193 and +65 to -117 in smooth-muscle cells. In contrast with the smooth-muscle cells, no CAT activity was detected in Rat-1 fibroblasts with the smallest two fragments. The residual promoter activity in the smallest fragment of the SM22.alpha. promoter tested suggested that, unlike the smooth-muscle .alpha.-actin promoter, transcription from the SM22.alpha. promoter can occur in smooth-muscle cells in the absence of factors binding to CC(A/Trich)6GG (CArG box) or CANNTG (E box) motifs.

L75 ANSWER 42 OF 46 CAPLUS COPYRIGHT 2000 ACS

1995:495839 Document No. 123:134745 A regulatory element downstream of the rat SM22.alpha. gene transcription start point enhances reporter gene expression in vascular smooth muscle cells. Osbourn, Jane K.; Weissberg, Peter L.; Shanahan, Catherine M. (University Department of Medicine, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK). Gene, 154(2), 249-53 (English) 1995. CODEN: GENED6. ISSN: 0378-1119.

AB SM22.alpha. is a 22-kDa protein of unknown function, the mRNA of which is highly and specifically expressed in smooth muscle cells (SMC). The 5' untranslated leader sequence of the rat SM22.alpha. gene was found to contain two introns of 3.6 and 2.9kb. Two transcripts of SM22.alpha. exist in all SMC types examd., and genomic mapping of the gene suggests these transcripts result from different 5' transcription start points, split by the 2.9-kb intron. A small intron (102bp), which contains an E-box consensus sequence, was found within the coding region 178bp from the ATG start codon. The 3.6-kb intron contains 82bp which show 98% homol. at the RNA level with the rat identifier sequence (ID). Transient reporter gene assays demonstrate that a 576-bp fragment, including the ID, contains a regulatory element which may contribute to the SMC-specific expression of SM22.alpha..

L75 ANSWER 43 OF 46 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 24

1994:647779 Document No. 121:247779 The Caenorhabditis elegans muscle-affecting gene unc-87 encodes a novel thin filament-associated protein. Goetinck, S.; Waterston, Robert H. (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). J. Cell Biol., 127(1), 79-93 (English) 1994. CODEN: JCLBA3. ISSN: 0021-9525.

AB Mutations in the unc-87 gene of Caenorhabditis elegans affect the structure and function of body-wall muscle, resulting in variable paralysis. We cloned the unc-87 gene by taking advantage of a transposon-induced allele of unc-87 and the correspondence of the genetic and phys. maps in C. elegans. A genomic clone was isolated that alleviates the mutant phenotype when introduced into unc-87 mutants. Sequence anal. of a corresponding cDNA clone predicts a 357-amino acid, 40-kD protein that is similar to portions of the vertebrate smooth muscle proteins calponin and **SM22alpha**, the Drosophila muscle protein mp20, the deduced product of the C. elegans cDNA cm7g3, and the rat neuronal protein np25. Anal. of the genomic sequence and of various transcripts represented in a cDNA library suggest that unc-87 mRNAs are subject to alternative splicing. Immunohistochem. of wild-type and mutant animals with antibodies to an unc-87 fusion protein indicates that the gene product is localized to the I-band of body-wall muscle. Studies of the UNC-87 protein in other muscle mutants suggest that the unc-87 gene product assocs. with thin filaments, in a manner that does not depend on the presence of the thin filament protein tropomyosin.

L75 ANSWER 44 OF 46 CAPLUS COPYRIGHT 2000 ACS
1993:618698 Document No. 119:218698 Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells. Shanahan, Catherine M.; Weissberg, Peter L.; Metcalfe, James C. (Dep. Biochem., Univ. Cambridge, Cambridge, CB2 1QW, UK). Circ. Res., 73(1), 193-204 (English) 1993. CODEN: CIRUAL. ISSN: 0009-7330.
AB To isolate specific markers of both differentiated and proliferating vascular smooth muscle cells (VSMCs), the authors used the technique of differential cDNA screening using RNA from cultured rat aortic VSMCs. The tissue specificity of expression of all of the cDNAs isolated was detd. by Northern anal. The authors isolated seven distinct cDNAs that were more strongly expressed in freshly dispersed, differentiated, aortic VSMCs than in dedifferentiated late-passage cells. These were the cDNAs for tropoelastin (a matrix protein), .alpha.-smooth muscle (SM) actin, .gamma.-SM actin, calponin, and phospholamban, which are all proteins assocd. with the contractile function of differentiated VSMCs, and SM22.alpha., a smooth muscle-specific protein of unknown function, and CHIP28, a putative membrane channel protein that is not highly expressed in other SM tissues and may therefore be a new VSMC marker. Two cDNAs that were expressed preferentially in late-passage dedifferentiated VSMCs were also isolated. These were the cDNAs for osteopontin and matrix Gla protein (MGP). Like CHIP28, MGP was strongly expressed in aortic VSMCs but not in other types of tissues contg. SM cells, suggesting that both have specific functions in vascular tissue. Osteopontin and MGP have both previously been isolated from developing bone. Their expression in proliferating VSMCs suggests that they may be involved in regulating the calcification that commonly occurs in vascular lesions. The set of cDNAs obtained extends the range of DNA probes that are available for identifying VSMCs and characterizing their phenotype in vivo by in situ hybridization. Therefore, they should aid in the anal. of gene expression during the development of vessel lesions.

L75 ANSWER 45 OF 46 CAPLUS COPYRIGHT 2000 ACS
1992:525596 Document No. 117:125596 Gene cloning and nucleotide sequence of SM22.alpha. from the chicken gizzard smooth muscle. Nishida, Wataru; Kitami, Yutaka; Abe, Masahiro; Hiwada, Kunio (Sch. Med., Ehime Univ., 791-02, Japan). Biochem. Int., 23(4), 663-8 (English) 1991. CODEN: BIINDF. ISSN: 0158-5231.
AB A full-length cDNA encoding SM22.alpha. from chicken gizzard smooth muscle was cloned and sequenced. Cloned cDNA had a total length of 1214bp and contained a single open reading frame which encodes 200 amino acids with a calcd. mol. wt. of 22,214. The predicted amino acid sequence was in complete agreement with the sequence detd. by J. R. Pearlstone et al. (1987) using Edman degrdn. method except for 2 addnl. residues, isoleucine and serine at the C-terminus.

L75 ANSWER 46 OF 46 CAPLUS COPYRIGHT 2000 ACS
1987:511335 Document No. 107:111335 Amino acid sequence of chicken gizzard smooth muscle SM22.alpha.. Pearlstone, Joyce R.; Weber, Marion; Lees-Miller, James P.; Carpenter, Michael R.; Smillie, Lawrence B. (Dep. Biochem., Univ. Alberta, Edmonton, AB, T6G 2H7, Can.). J. Biol. Chem., 262(13), 5985-91 (English) 1987. CODEN: JBCHA3. ISSN: 0021-9258.
AB The complete amino acid sequence of SM22.alpha., a novel and abundant

22-kilodalton protein from chicken gizzard smooth muscle, was detd. by a combination of automated and manual Edman degrdn. methods on fragments produced by suitable chem. and proteolytic cleavages. The protein consists of a single polypeptide chain of 197 residues, has a mol. wt. of 21,978, and a net charge of +4.5 at neutral pH. The pattern of alternating hydrophilic and hydrophobic regions throughout the length of SM23.alpha. is typical of a globular protein. The overall secondary structural anal., using several algorithms based on the sequence, predicts
.apprx.31% .alpha.-helix, 24% .beta.-sheet, 18% .beta.-turn, and 27% random coil. A search against the National Biomedical Research Foundation
Protein Sequence Databank (Washington) and GenBank (Los Alamos) failed to demonstrate significant similarity with any other protein of known sequence.

=> dis his

(FILE 'HOME' ENTERED AT 13:55:55 ON 19 JUN 2000)

FILE 'REGISTRY' ENTERED AT 13:58:57 ON 19 JUN 2000
E "SM22.ALPHA."/CN
L1 1 S "SM22+"/CN

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT 14:03:05 ON 19 JUN 2000
L2 87 FILE MEDLINE
L3 105 FILE CAPLUS
L4 105 FILE BIOSIS
L5 85 FILE EMBASE
L6 11 FILE WPIDS
TOTAL FOR ALL FILES
L7 393 S SM22?
L8 638 FILE MEDLINE
L9 633 FILE CAPLUS
L10 666 FILE BIOSIS
L11 611 FILE EMBASE
L12 19 FILE WPIDS
TOTAL FOR ALL FILES
L13 2567 S LATENT(L)((TRANSFORM? GROWTH FACTOR OR TGF)(W)(B OR BETA) OR
L14 336 FILE MEDLINE
L15 304 FILE CAPLUS
L16 314 FILE BIOSIS
L17 296 FILE EMBASE
L18 7 FILE WPIDS
TOTAL FOR ALL FILES
L19 1257 S L13(5A)(BIND? PROTEIN? OR INHIBITOR?)
L20 27 FILE MEDLINE
L21 36 FILE CAPLUS
L22 47 FILE BIOSIS
L23 26 FILE EMBASE
L24 3 FILE WPIDS
TOTAL FOR ALL FILES
L25 139 S (INTEGRIN? OR D12.776.543.750.705.408/CT OR D24.611.834.408/C
L26 18 FILE MEDLINE
L27 25 FILE CAPLUS
L28 16 FILE BIOSIS
L29 17 FILE EMBASE

```
L30             2 FILE WPIDS
     TOTAL FOR ALL FILES
L31            78 S LEF 1(5A)(B OR BETA)(W)CATENIN?
L32         21269 FILE MEDLINE
L33         11862 FILE CAPLUS
L34         21237 FILE BIOSIS
L35         18972 FILE EMBASE
L36           465 FILE WPIDS
     TOTAL FOR ALL FILES
L37         73805 S (NEURAL CREST OR A16.254.600/CT OR NEUROBLASTOMA? OR
C4.557.4
L38           521 FILE MEDLINE
L39           341 FILE CAPLUS
L40           608 FILE BIOSIS
L41           471 FILE EMBASE
L42            26 FILE WPIDS
     TOTAL FOR ALL FILES
L43          1967 S L37(L)(SMOOTH(1A)MUSCLE? OR MUSCLE? OR A2.633.570/CT OR
A10.6
L44           238 FILE MEDLINE
L45           146 FILE CAPLUS
L46           229 FILE BIOSIS
L47           223 FILE EMBASE
L48             5 FILE WPIDS
     TOTAL FOR ALL FILES
L49           841 S L43(L)DIFFER?
L50            31 FILE MEDLINE
L51            15 FILE CAPLUS
L52            31 FILE BIOSIS
L53            30 FILE EMBASE
L54             0 FILE WPIDS
     TOTAL FOR ALL FILES
L55           107 S L49(L)(G5.331.375/CT OR G5.331.370/CT OR PHENOTYP? OR
GENE(2A
L56             1 FILE MEDLINE
L57             1 FILE CAPLUS
L58             1 FILE BIOSIS
L59             1 FILE EMBASE
L60             0 FILE WPIDS
     TOTAL FOR ALL FILES
L61             4 S L7(L)L55
L62             1 DUP REM L61 (3 DUPLICATES REMOVED)
L63            25 FILE MEDLINE
L64            17 FILE CAPLUS
L65            30 FILE BIOSIS
L66             2 FILE EMBASE
L67             3 FILE WPIDS
     TOTAL FOR ALL FILES
L68            77 S SM22ALPHA
L69            24 FILE MEDLINE
L70            17 FILE CAPLUS
L71            29 FILE BIOSIS
L72             2 FILE EMBASE
L73             3 FILE WPIDS
     TOTAL FOR ALL FILES
L74            75 S L68 NOT L61
L75            46 DUP REM L74 (29 DUPLICATES REMOVED)

=> s l49(l)(l19 or l25)
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L44(L)(L14'
```

L76 1 FILE MEDLINE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L45(L)(L15'
L77 0 FILE CAPLUS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L46(L)(L16'
L78 1 FILE BIOSIS
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L47(L)(L17'
L79 0 FILE EMBASE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L48(L)(L18'
L80 1 FILE WPIDS

TOTAL FOR ALL FILES
L81 3 L49(L)(L19 OR L25)

=> s l81 not (l61 or l68)
L82 1 FILE MEDLINE
L83 0 FILE CAPLUS
L84 1 FILE BIOSIS
L85 0 FILE EMBASE
L86 0 FILE WPIDS

TOTAL FOR ALL FILES
L87 2 L81 NOT (L61 OR L68)

=> dup rem l87
PROCESSING COMPLETED FOR L87
L88 2 DUP REM L87 (0 DUPLICATES REMOVED)

=> d 1-2 cbib abs hit

L88 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2000 BIOSIS
1999:524403 Document No.: PREV199900524403. Identification of **latent transforming growth factor-beta binding protein** as a gene markedly induced during **neural crest** cell to **smooth muscle** cell **differentiation**. Watanabe, Masafumi; Layne, Matthew D.; Jain, Mukesh K.; Hsieh, Chung-Ming; Chin, Michael T.; Yet, Shaw-Fang; Lee, Mu-En. Harvard Sch. Public Health, Boston, MA USA. Circulation, (Oct. 27, 1998) Vol. 98, No. 17 SUPPL., pp. I605. Meeting Info.: 71st Scientific Sessions of the American Heart Association Dallas, Texas, USA November 8-11, 1998 The American Heart Association. ISSN: 0009-7322. Language: English.

TI Identification of **latent transforming growth factor-beta binding protein** as a gene markedly induced during **neural crest** cell to **smooth muscle** cell **differentiation.**

IT Major Concepts
Cardiovascular System (Transport and Circulation); Development; Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Parts, Structures, & Systems of Organisms
neural crest cell: **differentiation**, embryonic structure, nervous system; vascular **smooth muscle** cell: circulatory system, muscular system, **differentiation**

IT Chemicals & Biochemicals
LTBP gene; TGF-beta [transforming growth factor-beta]: regulation

L88 ANSWER 2 OF 2 MEDLINE
1998407806 Document Number: 98407806. ILK (beta1-integrin-linked protein kinase): a novel immunohistochemical marker for Ewing's sarcoma and primitive neuroectodermal tumour. Chung D H; Lee J I; Kook M C; Kim J R; Kim S H; Choi E Y; Park S H; Song H G. (Department of Pathology, Seoul National University College of Medicine, Korea.) VIRCHOWS ARCHIV, (1998 Aug) 433 (2) 113-7. Journal code: BZD. ISSN: 0945-6317. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB ILK (beta1-integrin-linked protein kinase) is a recently identified 59-kDa serine/threonine protein kinase that interacts with the cytoplasmic domain of the beta1-integrin containing four ankyrin-like repeats. We have developed a polyclonal antibody against ILK and explored the ILK immunoreactivity in normal human cells and tissues. ILK was mainly expressed in cardiac **muscle** and skeletal **muscles**. Surprisingly, ILK expression was observed in Ewing's sarcoma (ES; 100%), primitive neuroectodermal tumour (PNET; 100%), medulloblastoma (100%), and **neuroblastoma** (33.3%), whereas other small round cell sarcomas were not stained by the anti-ILK antibody. These results suggest that ILK could be a novel marker for tumours with primitive neural **differentiation**. Our findings support the notion that ES is a tumour that is closely related to PNET and that both originate from the neuroectoderm. ILK may be a sensitive and specific immunohistochemical marker and useful for the positive identification of ES and PNET in formalin-fixed, paraffin-embedded tissue sections.

AB ILK (beta1-integrin-linked protein kinase) is a recently identified 59-kDa serine/threonine protein kinase that interacts with the cytoplasmic domain of the beta1-integrin containing four ankyrin-like repeats. We have developed a polyclonal antibody against ILK and explored the ILK immunoreactivity in normal human cells and tissues. ILK was mainly expressed in cardiac **muscle** and skeletal **muscles**. Surprisingly, ILK expression was observed in Ewing's sarcoma (ES; 100%), primitive neuroectodermal tumour (PNET; 100%), medulloblastoma (100%), and **neuroblastoma** (33.3%), whereas other small round cell sarcomas were not stained by the anti-ILK antibody. These results suggest that ILK could be a novel marker for tumours with primitive neural **differentiation**. Our findings support the notion that ES is a tumour that is closely related to PNET and that both originate from the neuroectoderm. ILK may be a sensitive and specific immunohistochemical marker and useful for the positive identification of ES and PNET in formalin-fixed, paraffin-embedded tissue sections.

CN EC 2.7.10 (Protein-Serine-Threonine Kinases); EC 2.7.10.- (**integrin-linked kinase**); 0 (Tumor Markers, Biological)

=> s l49(l)(ilk or aortic carboxypeptid? or torsin? or cct? or prothymosin? or limk2 or cca or confluent 3yi or 3yi cell associat? or interferon? or internex? or caspase? or ahnak or desmoyokin? or tsc36 or tsc 36 or transcobalamin?)

L89	2	FILE MEDLINE
L90	3	FILE CAPLUS
L91	2	FILE BIOSIS
L92	2	FILE EMBASE
L93	0	FILE WPIDS

TOTAL FOR ALL FILES
L94 9 L49(L)(ILK OR AORTIC CARBOXYPEPTID? OR TORSIN? OR CCT? OR PROTHY
MOSIN? OR LIMK2 OR CCA OR CONFLUENT 3YI OR 3YI CELL ASSOCIAT? OR INTERFERON? OR INTERNEX? OR CASPASE? OR AHNAK OR DESMOYOKIN?
OR TSC36 OR TSC 36 OR TRANSCOBALAMIN?)

=> dup rem l94
PROCESSING COMPLETED FOR L94
L95 3 DUP REM L94 (6 DUPLICATES REMOVED)

=> d cbib abs 1-3 hit

L95 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2000 ACS
1999:299499 Document No. 130:322697 An in vitro system for inducing neural crest cell differentiation to vascular smooth muscle cells and its use in identifying regulators and their genes. (President and Fellows of Harvard College, USA; Lee, Arthur M. E.; Jain, Mukesh; Watanabe, Masafumi). PCT Int. Appl. WO 9921965 A2 19990506, 92 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US22897 19981028. PRIORITY: US 1997-63363 19971028; US 1998-80420 19980402; US 1998-96685 19980814.

AB This invention is directed to an in vitro system for rapidly and uniformly inducing immortalized neural crest cells to differentiate to vascular smooth muscle cells. As excessive proliferation of vascular smooth muscle cells is a phenotypic response to the development of occlusive arteriosclerotic disease, the in vitro system of this invention is used to identify mol. regulators of smooth muscle cell development and differentiation. As the mol. regulators of smooth muscle cell differentiation are identified, the invention also encompasses methods to isolate the genes coding for these regulators. This invention also relates to mols. identified through the use of the invention's in vitro system, as well as to compds. that inhibit or regulate the identified mols. Neural crest Monc-1 cells were induced to differentiate to vascular smooth muscle cells by application of a smooth muscle cell differentiation medium (SMDM) contg. inorg. salts, amino acids, vitamins, and other components (specific components are listed) supplemented with 10% fetal bovine serum, penicillin, streptomycin and Hepes (pH 7.4).

IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**Cca** (confluent 3Y1 cell-assocd.), as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Chaperonins
RL: MSC (Miscellaneous)
(TCP-1 complex contg., **cct**.zeta. subunit of, as gene product

affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Proteins (specific proteins and subclasses)
RL: MSC (Miscellaneous)
(TCP-1, chaperonin-contg., **cct**.zeta. subunit of, as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**TSC-36** (TGF-inducible protein), as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Mouse
(**aortic carboxypeptidase**-like protein (ACLP) of human and; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**desmoyokins**, as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**interferon**-induced, as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**internexins**, as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Protein sequences
(of **aortic carboxypeptidase**-like protein (ACLP) of human and mouse; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT **Interferons**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(protein activatable by, as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Antibodies
RL: BPN (Biosynthetic preparation); BPR (Biological process); BIOL (Biological study); PREP (Preparation); PROC (Process)

(to **aortic carboxypeptidase**-like protein (ACLP), prepn. of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT Proteins (specific proteins and subclasses)
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**torsin**, as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT 196718-43-1P, Protein ACLP (**aortic carboxypeptidase**-like protein) (mouse precursor) 210978-43-1P, Protein ACLP (**aortic carboxypeptidase**-like protein) (human precursor)
RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); PROC (Process)
(amino acid sequence; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

IT 12774-24-2, **Transcobalamin** 24305-27-9, Thyrotropin-releasing hormone 86480-67-3, Ubiquitin carboxyl-terminal hydrolase 89964-14-7, **Prothymosin** .alpha. 172306-54-6, LIM-kinase 2 186322-81-6, **Caspase**
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(as gene product affected, identification of; in vitro system for inducing **neural crest** cell **differentiation** to vascular **smooth muscle** cells and its use in identifying regulators and their genes)

L95 ANSWER 2 OF 3 MEDLINE DUPLICATE 1
1998288305 Document Number: 98288305. Aortic carboxypeptidase-like protein, a novel protein with discoidin and carboxypeptidase-like domains, is up-regulated during vascular smooth muscle cell differentiation. Layne M D; Endege W O; Jain M K; Yet S F; Hsieh C M; Chin M T; Perrella M A; Blanar M A; Haber E; Lee M E. (Cardiovascular Biology Laboratory, Harvard School of Public Health, Boston, Massachusetts 02115, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 19) 273 (25) 15654-60. Journal code: HIV.
ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Phenotypic modulation of vascular **smooth muscle** cells plays an important role in the pathogenesis of arteriosclerosis. In a screen of proteins expressed in human aortic **smooth muscle** cells, we identified a novel gene product designated **aortic carboxypeptidase**-like protein (ACLP). The approximately 4-kilobase human cDNA and its mouse homologue encode 1158 and 1128 amino acid proteins, respectively, that are 85% identical. ACLP is a nonnuclear protein that contains a signal peptide, a lysine- and proline-rich 11-amino acid repeating motif, a discoidin-like domain, and a C-terminal domain with 39% identity to carboxypeptidase E. By Western blot analysis and in situ hybridization, we detected abundant ACLP expression in the adult aorta. ACLP was expressed predominantly in the **smooth muscle** cells of the adult mouse aorta but not in the adventitia or in several other tissues. In cultured mouse aortic **smooth muscle** cells, ACLP mRNA and protein were up-regulated 2-3-fold

after serum starvation. Using a recently developed **neural crest** cell to **smooth muscle** cell in vitro **differentiation** system, we found that ACLP mRNA and protein were not expressed in **neural crest** cells but were up-regulated dramatically with the **differentiation** of these cells. These results indicate that ACLP may play a role in **differentiated** vascular **smooth muscle** cells.

AB Phenotypic modulation of vascular **smooth muscle** cells plays an important role in the pathogenesis of arteriosclerosis. In a screen of proteins expressed in human aortic **smooth muscle** cells, we identified a novel gene product designated **aortic carboxypeptidase**-like protein (ACLP). The approximately 4-kilobase human cDNA and its mouse homologue encode 1158 and 1128 amino acid proteins, respectively, that are 85% identical. ACLP is a nonnuclear protein that contains a signal peptide, a lysine- and proline-rich 11-amino acid repeating motif, a discoidin-like domain, and a C-terminal domain with 39% identity to carboxypeptidase E. By Western blot analysis and in situ hybridization, we detected abundant ACLP expression in the adult aorta. ACLP was expressed predominantly in the **smooth muscle** cells of the adult mouse aorta but not in the adventitia or in several other tissues. In cultured mouse aortic **smooth muscle** cells, ACLP mRNA and protein were up-regulated 2-3-fold after serum starvation. Using a recently developed **neural crest** cell to **smooth muscle** cell in vitro **differentiation** system, we found that ACLP mRNA and protein were not expressed in **neural crest** cells but were up-regulated dramatically with the **differentiation** of these cells. These results indicate that ACLP may play a role in **differentiated** vascular **smooth muscle** cells.

L95 ANSWER 3 OF 3 MEDLINE DUPLICATE 2

1998407806 Document Number: 98407806. ILK (beta1-integrin-linked protein kinase): a novel immunohistochemical marker for Ewing's sarcoma and primitive neuroectodermal tumour. Chung D H; Lee J I; Kook M C; Kim J R; Kim S H; Choi E Y; Park S H; Song H G. (Department of Pathology, Seoul National University College of Medicine, Korea.) VIRCHOWS ARCHIV, (1998 Aug) 433 (2) 113-7. Journal code: BZD. ISSN: 0945-6317. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB **ILK** (beta1-integrin-linked protein kinase) is a recently identified 59-kDa serine/threonine protein kinase that interacts with the cytoplasmic domain of the beta1-integrin containing four ankyrin-like repeats. We have developed a polyclonal antibody against **ILK** and explored the **ILK** immunoreactivity in normal human cells and tissues. **ILK** was mainly expressed in cardiac **muscle** and skeletal **muscles**. Surprisingly, **ILK** expression was observed in Ewing's sarcoma (ES; 100%), primitive neuroectodermal tumour (PNET; 100%), medulloblastoma (100%), and **neuroblastoma** (33.3%), whereas other small round cell sarcomas were not stained by the anti-**ILK** antibody. These results suggest that **ILK** could be a novel marker for tumours with primitive neural **differentiation**. Our findings support the notion that ES is a tumour that is closely related to PNET and that both originate from the neuroectoderm. **ILK** may be a sensitive and specific immunohistochemical marker and useful for the positive identification of ES and PNET in formalin-fixed, paraffin-embedded tissue sections.

AB **ILK** (beta1-integrin-linked protein kinase) is a recently identified 59-kDa serine/threonine protein kinase that interacts with the cytoplasmic domain of the beta1-integrin containing four ankyrin-like repeats. We have developed a polyclonal antibody against **ILK** and

explored the **ILK** immunoreactivity in normal human cells and tissues. **ILK** was mainly expressed in cardiac **muscle** and skeletal **muscles**. Surprisingly, **ILK** expression was observed in Ewing's sarcoma (ES; 100%), primitive neuroectodermal tumour (PNET; 100%), medulloblastoma (100%), and **neuroblastoma** (33.3%), whereas other small round cell sarcomas were not stained by the anti-**ILK** antibody. These results suggest that **ILK** could be a novel marker for tumours with primitive neural **differentiation**. Our findings support the notion that ES is a tumour that is closely related to PNET and that both originate from the neuroectoderm. **ILK** may be a sensitive and specific immunohistochemical marker and useful for the positive identification of ES and PNET in formalin-fixed, paraffin-embedded tissue sections.

```
=> s l49(l)(dos or epididy? or "e1" precursor or ubiquitin carboxy? or
hydrolase or thyrotropin releas? or decorin)
L96           4 FILE MEDLINE
L97           6 FILE CAPLUS
L98           5 FILE BIOSIS
L99           3 FILE EMBASE
L100          0 FILE WPIDS

TOTAL FOR ALL FILES
L101         18 L49(L)(DOS OR EPIDIDY? OR "E1" PRECURSOR OR UBIQUITIN CARBOXY?
                OR HYDROLASE OR THYROTROPIN RELEAS? OR DECORIN)

=> s l101 not l94
L102          4 FILE MEDLINE
L103          5 FILE CAPLUS
L104          5 FILE BIOSIS
L105          3 FILE EMBASE
L106          0 FILE WPIDS

TOTAL FOR ALL FILES
L107         17 L101 NOT L94

=> dup rem l107
PROCESSING COMPLETED FOR L107
L108           5 DUP REM L107 (12 DUPLICATES REMOVED)

=> d 1-5 cbib abs hit

L108 ANSWER 1 OF 5  MEDLINE                              DUPLICATE 1
1998175885  Document Number: 98175885.   Xenopus eHAND: a marker for the
     developing cardiovascular system of the embryo that is regulated by bone
     morphogenetic proteins. Sparrow D B; Kotecha S; Towers N; Mohun T J.
     (Division of Developmental Biology, National Institute for Medical
     Research, London, UK. ) MECHANISMS OF DEVELOPMENT, (1998 Feb) 71 (1-2)
     151-63.  Journal code: AXF. ISSN: 0925-4773. Pub. country: Ireland.
     Language: English.
AB   The bHLH protein eHAND is a sensitive marker for cardiovascular
precursors
     in the Xenopus embryo. The earliest site of expression is a broad domain
     within the lateral plate mesoderm of the tailbud embryo. This domain
     comprises precursors that contribute to the posterior cardinal veins in
     later stages. Surprisingly, expression is profoundly asymmetric at this
     stage and is random with respect to embryo side. XeHAND is also expressed
     in an anterior domain that encompasses the prospective heart region.
     Within the myocardium and pericardium, transcripts are also
asymmetrically
```

distributed, but in these tissues they are localised in a left-sided manner. Later in development XeHAND transcripts are largely restricted to the ventral aorta, aortic arches and venous inflow tract (sinus venosus) which flank the heart itself, but no expression is detected in **neural crest** derivatives at any stage. This demonstrates that patterns of XeHAND expression **differ** markedly amongst vertebrates and that in Xenopus, XeHAND expression identifies all of the earliest formed elements of the cardiovascular system. In animal cap explants, expression of XeHAND (but not other markers of cardiogenic **differentiation**) is strongly induced by ectopic expression of the TGFbeta family members, BMP-2 and BMP-4, but this can be blocked by coexpression of a dominant negative BMP receptor. This suggests that XeHAND expression in the embryo is regulated by the ventralising signals of bone morphogenetic proteins. High levels of expression are also detected in explants treated with high **doses** of activin A which induces cardiac **muscle differentiation**. No such effect is seen with lower **doses** of activin, indicating that a second pathway may regulate the XeHAND gene during cardiogenesis.

AB The bHLH protein eHAND is a sensitive marker for cardiovascular precursors in the Xenopus embryo. The earliest site of expression is a broad domain within the lateral plate mesoderm of the tailbud embryo. This domain comprises precursors that contribute to the posterior cardinal veins in later stages. Surprisingly, expression is profoundly asymmetric at this stage and is random with respect to embryo side. XeHAND is also expressed in an anterior domain that encompasses the prospective heart region. Within the myocardium and pericardium, transcripts are also asymmetrically distributed, but in these tissues they are localised in a left-sided manner. Later in development XeHAND transcripts are largely restricted to the ventral aorta, aortic arches and venous inflow tract (sinus venosus) which flank the heart itself, but no expression is detected in **neural crest** derivatives at any stage. This demonstrates that patterns of XeHAND expression **differ** markedly amongst vertebrates and that in Xenopus, XeHAND expression identifies all of the earliest formed elements of the cardiovascular system. In animal cap explants, expression of XeHAND (but not other markers of cardiogenic **differentiation**) is strongly induced by ectopic expression of the TGFbeta family members, BMP-2 and BMP-4, but this can be blocked by coexpression of a dominant negative BMP receptor. This suggests that XeHAND expression in the embryo is regulated by the ventralising signals of bone morphogenetic proteins. High levels of expression are also detected in explants treated with high **doses** of activin A which induces cardiac **muscle differentiation**. No such effect is seen with lower **doses** of activin, indicating that a second pathway may regulate the XeHAND gene during cardiogenesis.

L108 ANSWER 2 OF 5 MEDLINE DUPLICATE 2

97470973 Document Number: 97470973. Integration of multiple instructive cues by neural crest stem cells reveals cell-intrinsic biases in relative growth factor responsiveness. Shah N M; Anderson D J. (Division of Biology 216-76, Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Oct 14) 94 (21) 11369-74. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Growth factors can influence lineage determination of **neural crest** stem cells (NCSCs) in an instructive manner, in vitro.

Because NCSCs are likely exposed to multiple signals in vivo, these findings raise the question of how stem cells would integrate such combined influences. Bone morphogenetic protein 2 (BMP2) promotes neuronal **differentiation** and glial growth factor 2 (GGF2) promotes glial **differentiation**; if NCSCs are exposed to saturating concentrations of both factors, BMP2 appears dominant. By contrast, if the cells are exposed to saturating concentrations of both BMP2 and transforming growth factor beta1 (which promotes **smooth muscle differentiation**), the two factors appear codominant. Sequential addition experiments indicate that NCSCs require 48-96 hrs in GGF2 before they commit to a glial fate, whereas the cells commit to a **smooth muscle** fate within 24 hr in transforming growth factor beta1. The delayed response to GGF2 does not reflect a lack of functional receptors; however, because the growth factor induces rapid mitogen-activated protein kinase phosphorylation in naive cells. Furthermore, GGF2 can attenuate induction of the neurogenic transcription factor mammalian achaete-scute homolog 1, by low **doses** of BMP2. This short-term antineurogenic influence of GGF2 is not sufficient for glial lineage commitment, however.

These data imply that NCSCs exhibit cell-intrinsic biases in the timing and relative dosage sensitivity of their responses to instructive factors that influence the outcome of lineage decisions in the presence of multiple factors. The relative delay in glial lineage commitment, moreover, apparently reflects successive short-term and longer-term actions of GGF2. Such a delay may help to explain why glia normally **differentiate** after neurons, in vivo.

AB Growth factors can influence lineage determination of **neural crest** stem cells (NCSCs) in an instructive manner, in vitro. Because NCSCs are likely exposed to multiple signals in vivo, these findings raise the question of how stem cells would integrate such combined influences. Bone morphogenetic protein 2 (BMP2) promotes neuronal **differentiation** and glial growth factor 2 (GGF2) promotes glial **differentiation**; if NCSCs are exposed to saturating concentrations of both factors, BMP2 appears dominant. By contrast, if the cells are exposed to saturating concentrations of both BMP2 and transforming growth factor beta1 (which promotes **smooth muscle differentiation**), the two factors appear codominant. Sequential addition experiments indicate that NCSCs require 48-96 hrs in GGF2 before they commit to a glial fate, whereas the cells commit to a **smooth muscle** fate within 24 hr in transforming growth factor beta1. The delayed response to GGF2 does not reflect a lack of functional receptors; however, because the growth factor induces rapid mitogen-activated protein kinase phosphorylation in naive cells. Furthermore, GGF2 can attenuate induction of the neurogenic transcription factor mammalian achaete-scute homolog 1, by low **doses** of BMP2. This short-term antineurogenic influence of GGF2 is not sufficient for glial lineage commitment, however.

These data imply that NCSCs exhibit cell-intrinsic biases in the timing and relative dosage sensitivity of their responses to instructive factors that influence the outcome of lineage decisions in the presence of multiple factors. The relative delay in glial lineage commitment, moreover, apparently reflects successive short-term and longer-term actions of GGF2. Such a delay may help to explain why glia normally **differentiate** after neurons, in vivo.

L108 ANSWER 3 OF 5 MEDLINE DUPLICATE 3
1998071511 Document Number: 98071511. Retinoic acid-induced asymmetric

craniofacial growth and cleft palate in the TO mouse fetus. Padmanabhan R;
Ahmed I. (Department of Anatomy, Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.) REPRODUCTIVE TOXICOLOGY, (1997 Nov-Dec) 11 (6) 843-60. Journal code: BE4. ISSN: 0890-6238. Pub. country: United States. Language: English.

AB The etiology and pathogenetic mechanisms of cleft palate (CP) are rather uncertain. Both genetic and environmental factors are known to cause failure of horizontalization and/or failure of fusion of the palatal shelves resulting in CP. Retinoic acid (RA)-induced CP in the mouse is reported to exhibit two peaks of incidence separated by a less sensitive window. The morphologic bases of the **differential** sensitivity are not known. The objectives of this study were to determine whether the TO mouse had similar peaks of sensitivity to RA-induced CP, and if it did, to evaluate the morphologic and histologic bases of CP induced at an early [Gestation Day (GD) 8] and at a late (GD 12) stage of embryonic development. Single **doses** of all-trans-RA were administered to groups of mice on one of GD 8 to 15. On GD 18, fetuses were evaluated for the presence of CP, and the developmental stage of the palatal shelves was determined. All **doses** of RA were found to induce a high incidence of CP in the GD 8 to 13 treatment groups. GD 14 and 15 were not susceptible. There were no stage-dependent peaks or less sensitive windows, indicating that RA-induced CP in this strain is a continuum from GD 8 through 13. Morphologically clefting in the GD 8-RA treatment group was characterized by extreme hypoplasia (65% to 100%, depending on the dose) or agenesis (35% in the 200 mg/kg group) of the palatal shelves and associated with astomia, microstomia, aglossia, microglossia, and micrognathia with fusion of mandible, maxilla, and zygoma. Treatment on subsequent days of gestation resulted in CP with the shelves reaching progressively higher levels of maturity in terms of developmental staging.
There was no case of CP with horizontalized shelves apposing but failing to fuse with each other. The facial skeleton of GD 12-RA group was hypoplastic but not malformed. Reduction in all dimensions of the cranium and mandible was highly significant ($P < 0.001$) in the GD 8-RA group, whereas there was a clear imbalance between the vertical growth and that in other directions in the GD 12-RA group. The CR length, head and body weights, and the protein content of heads of GD 8-RA-treated embryos were significantly reduced. Histologic studies showed that both the intrinsic and extrinsic **muscles** of the tongue and face, growth of the Meckel's cartilage, and ossification of the mandible were severely affected in the GD 8 treatment group, whereas these tissues were only moderately affected in the embryos of the GD 12-RA group. However, the quality of cytodifferentiation of the **muscles** was not affected in either group. These data provide evidence for the susceptibility continuum of CP in this strain. They also indicate that agenesis and hypoplasia of the palatal shelves and primordia of craniofacial skeleton and musculature contribute to CP, the relative involvement of the components depending on the stage of drug administration. In the absence of pronounced cell death, it appears that RA possibly produces its deleterious effects on the precursors of craniofacial primordia, such as the **neural crest**, by misexpression of developmentally important genes.

AB The etiology and pathogenetic mechanisms of cleft palate (CP) are rather uncertain. Both genetic and environmental factors are known to cause failure of horizontalization and/or failure of fusion of the palatal shelves resulting in CP. Retinoic acid (RA)-induced CP in the mouse is reported to exhibit two peaks of incidence separated by a less sensitive

window. The morphologic bases of the **differential** sensitivity are not known. The objectives of this study were to determine whether the TO mouse had similar peaks of sensitivity to RA-induced CP, and if it did, to evaluate the morphologic and histologic bases of CP induced at an early [Gestation Day (GD) 8] and at a late (GD 12) stage of embryonic development. Single **doses** of all-trans-RA were administered to groups of mice on one of GD 8 to 15. On GD 18, fetuses were evaluated for the presence of CP, and the developmental stage of the palatal shelves was determined. All **doses** of RA were found to induce a high incidence of CP in the GD 8 to 13 treatment groups. GD 14 and 15 were not susceptible. There were no stage-dependent peaks or less sensitive windows, indicating that RA-induced CP in this strain is a continuum from GD 8 through 13. Morphologically clefting in the GD 8-RA treatment group was characterized by extreme hypoplasia (65% to 100%, depending on the dose) or agenesis (35% in the 200 mg/kg group) of the palatal shelves and associated with astomia, microstomia, aglossia, microglossia, and micrognathia with fusion of mandible, maxilla, and zygoma. Treatment on subsequent days of gestation resulted in CP with the shelves reaching progressively higher levels of maturity in terms of developmental staging.

There was no case of CP with horizontalized shelves apposing but failing to fuse with each other. The facial skeleton of GD 12-RA group was hypoplastic but not malformed. Reduction in all dimensions of the cranium and mandible was highly significant ($P < 0.001$) in the GD 8-RA group, whereas there was a clear imbalance between the vertical growth and that in other directions in the GD 12-RA group. The CR length, head and body weights, and the protein content of heads of GD 8-RA-treated embryos were significantly reduced. Histologic studies showed that both the intrinsic and extrinsic **muscles** of the tongue and face, growth of the Meckel's cartilage, and ossification of the mandible were severely affected in the GD 8 treatment group, whereas these tissues were only moderately affected in the embryos of the GD 12-RA group. However, the quality of cytodifferentiation of the **muscles** was not affected in either group. These data provide evidence for the susceptibility continuum of CP in this strain. They also indicate that agenesis and hypoplasia of the palatal shelves and primordia of craniofacial skeleton and musculature contribute to CP, the relative involvement of the components depending on the stage of drug administration. In the absence of pronounced cell death, it appears that RA possibly produces its deleterious effects on the precursors of craniofacial primordia, such as the **neural crest**, by misexpression of developmentally important genes.

L108 ANSWER 4 OF 5 MEDLINE DUPLICATE 4

95253075 Document Number: 95253075. Calculated and TLD-based absorbed dose estimates for I-131-labeled 3F8 monoclonal antibody in a human neuroblastoma xenograft nude mouse model. Ugur O; Scott A M; Kostakoglu L; Hui T E; Masterson M E; Febo R; Sgouros G; Rosa E; Mehta B M; Fisher D R; et al. (Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.) NUCLEAR MEDICINE AND BIOLOGY, (1995 Jan) 22 (1) 87-93. Journal code: BOO. ISSN: 0969-8051. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Preclinical evaluation of the therapeutic potential of radiolabeled antibodies is commonly performed in a xenografted nude mouse model. To assess therapeutic efficacy it is important to estimate the absorbed dose to the tumor and normal tissues of the nude mouse. The current study was

designed to accurately measure radiation does to human **neuroblastoma** xenografts and normal organs in nude mice treated with I-131-labeled 3F8 monoclonal antibody (MoAb) against disialoganglioside GD2 antigen. Absorbed dose estimates were obtained using two **different** approaches: (1) measurement with teflon-imbedded CaSO4:Dy mini-thermoluminescent dosimeters (TLDs) and (2) calculations using mouse S-factors. The calculated total dose to tumor one week after i.v. injection of the 50 microCi I-131-3F8 MoAb was 604 cGy. The corresponding decay corrected and not corrected TLD measurements were 109 +/- 9 and 48.7 +/- 3.4 cGy respectively. The calculated to TLD-derived dose ratios for tumor ranged from 6.1 at 24 h to 5.5 at 1 week. The light output fading rate was found to depend upon the tissue type within which the TLDs were implanted. The decay rate in tumor, **muscle**, subcutaneous tissue and in vitro, were 9.5, 5.0, 3.7 and 0.67% per day, respectively. We have demonstrated that the type of tissue in which the TLD was implanted strongly influenced the in vivo decay of light output. Even with decay correction, a significant discrepancy was observed between MIRD-based calculated and CaSO4:Dy mini-TLD measured absorbed **doses**. Batch dependence, pH of the tumor or other variables associated with TLDs which are not as yet well known may account for this discrepancy.

AB Preclinical evaluation of the therapeutic potential of radiolabeled antibodies is commonly performed in a xenografted nude mouse model. To assess therapeutic efficacy it is important to estimate the absorbed dose to the tumor and normal tissues of the nude mouse. The current study was designed to accurately measure radiation does to human **neuroblastoma** xenografts and normal organs in nude mice treated with I-131-labeled 3F8 monoclonal antibody (MoAb) against disialoganglioside GD2 antigen. Absorbed dose estimates were obtained using two **different** approaches: (1) measurement with teflon-imbedded CaSO4:Dy mini-thermoluminescent dosimeters (TLDs) and (2) calculations using mouse S-factors. The calculated total dose to tumor one week after i.v. injection of the 50 microCi I-131-3F8 MoAb was 604 cGy. The corresponding decay corrected and not corrected TLD measurements were 109 +/- 9 and 48.7 +/- 3.4 cGy respectively. The calculated to TLD-derived dose ratios for tumor ranged from 6.1 at 24 h to 5.5 at 1 week. The light output fading rate was found to depend upon the tissue type within which the TLDs were implanted. The decay rate in tumor, **muscle**, subcutaneous tissue and in vitro, were 9.5, 5.0, 3.7 and 0.67% per day, respectively. We have demonstrated that the type of tissue in which the TLD was implanted strongly influenced the in vivo decay of light output. Even with decay correction, a significant discrepancy was observed between MIRD-based calculated and CaSO4:Dy mini-TLD measured absorbed **doses**. Batch dependence, pH of the tumor or other variables associated with TLDs which are not as yet well known may account for this discrepancy.

L108 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 5

1994:240669 Document No. 120:240669 Basic fibroblast growth factor induces differentiation of neural tube and neural crest lineages of cultured ectoderm cells from Xenopus gastrula. Kengaku, Mineko; Okamoto, Harumasa (Fac. Med., Univ. Tokyo, Tokyo, 113, Japan). Development (Cambridge, UK), 119(4), 1067-78 (English) 1993. CODEN: DEVPED. ISSN: 0950-1991.

AB The vertebrate nervous system is initially induced from a section of

dorsal ectoderm by signal(s) from the underlying dorsal mesoderm during gastrulation. In an effort to identify the neural inducing factor(s) emanating from the dorsal mesoderm, the authors have examd. the inductive action of various growth factors by applying them to ectoderm cells from Xenopus gastrulae (8-12.5-h age; embryonic stage 9+ to 11 1/2) in a microculture system. Monoclonal antibodies that specifically recognize cellular **differentiation** antigens from three distinct ectoderm lineages (N1 for CNS neurons from neural tube, Mel for melanophores from **neural crest** and E3 for skin epidermal cells from epidermal lineages, resp.) and a mesoderm lineage (Mu1 for **muscle** cells) were used as markers to monitor the **differentiation** of cultured ectoderm cells. The authors found that basic fibroblast growth factor (bFGF) was capable of specifically and reproducibly inducing gastrula ectoderm cells to produce CNS neurons and melanophores at concns.
as low as 5 pM, a value about 50-fold lower than that required to induce the formation of **muscle** cells from blastula animal cap cells (6-h age; stage 8+). The induction of neural lineages by bFGF was correlated with a suppression of epidermal **differentiation** in a dose-dependent manner. The bFGF never induced the formation of **muscle** cells from gastrula ectoderm cells even at concns. as high as 5 nM. The response of ectoderm cells to bFGF changed dramatically during gastrulation. Ectoderm cells from early (8- to 9-h age; stage 9+ to 10) gastrula gave rise to CNS neurons, but yielded few melanophores. As ectoderm cells were prepd. from gastrulae of increasing age, their competence to form neurons was gradually lost, whereas the prodn. of melanophores was enhanced and peaked in 11-h gastrula (stage 101/2). The ability to form both neurons and melanophores was substantially reduced in
12.5-h gastrula (stage 111/2). By examg. ectoderm cells from the ventral and dorsal sides independently, it was also shown that during gastrulation
the change in response to bFGF of the ventral ectoderm preceded that of the dorsal ectoderm. The state of competence of the ectoderm changed primarily due to intrinsic factors rather than by instruction from other parts of the gastrula embryo. This was shown by adding bFGF to cultures of ectoderm cells that were isolated at 9-h (stage 10) and cultured for increasing periods to allow their autonomous development. The time course
of both loss of neuronal competence and gain and loss of melanophore competence closely paralleled that obsd. in vivo during gastrulation. Further, the authors showed that the sensitivity of ectoderm cells to bFGF
in the neuronal and melanophore induction processes decreased during later
gastrula stages; i.e., higher **doses** of bFGF were required for older ectoderm cells to be fully induced. A possible role of bFGF in neural induction during normal development is discussed.

AB The vertebrate nervous system is initially induced from a section of dorsal ectoderm by signal(s) from the underlying dorsal mesoderm during gastrulation. In an effort to identify the neural inducing factor(s) emanating from the dorsal mesoderm, the authors have examd. the inductive action of various growth factors by applying them to ectoderm cells from Xenopus gastrulae (8-12.5-h age; embryonic stage 9+ to 11 1/2) in a microculture system. Monoclonal antibodies that specifically recognize cellular **differentiation** antigens from three distinct ectoderm lineages (N1 for CNS neurons from neural tube, Mel for melanophores from **neural crest** and E3 for skin epidermal cells from epidermal lineages, resp.) and a mesoderm lineage (Mu1 for **muscle** cells) were used as markers to monitor the **differentiation** of cultured ectoderm cells. The authors found that basic fibroblast growth

factor (bFGF) was capable of specifically and reproducibly inducing gastrula ectoderm cells to produce CNS neurons and melanophores at concns.
as low as 5 pM, a value about 50-fold lower than that required to induce the formation of **muscle** cells from blastula animal cap cells (6-h age; stage 8+). The induction of neural lineages by bFGF was correlated with a suppression of epidermal **differentiation** in a dose-dependent manner. The bFGF never induced the formation of **muscle** cells from gastrula ectoderm cells even at concns. as high as 5 nM. The response of ectoderm cells to bFGF changed dramatically during gastrulation. Ectoderm cells from early (8- to 9-h age; stage 9+ to 10) gastrula gave rise to CNS neurons, but yielded few melanophores. As ectoderm cells were prepd. from gastrulae of increasing age, their competence to form neurons was gradually lost, whereas the prodn. of melanophores was enhanced and peaked in 11-h gastrula (stage 101/2). The ability to form both neurons and melanophores was substantially reduced in
12.5-h gastrula (stage 111/2). By examg. ectoderm cells from the ventral and dorsal sides independently, it was also shown that during gastrulation
the change in response to bFGF of the ventral ectoderm preceded that of the dorsal ectoderm. The state of competence of the ectoderm changed primarily due to intrinsic factors rather than by instruction from other parts of the gastrula embryo. This was shown by adding bFGF to cultures of ectoderm cells that were isolated at 9-h (stage 10) and cultured for increasing periods to allow their autonomous development. The time course
of both loss of neuronal competence and gain and loss of melanophore competence closely paralleled that obsd. in vivo during gastrulation. Further, the authors showed that the sensitivity of ectoderm cells to bFGF
in the neuronal and melanophore induction processes decreased during later
gastrula stages; i.e., higher **doses** of bFGF were required for older ectoderm cells to be fully induced. A possible role of bFGF in neural induction during normal development is discussed.

```
=> s lef(w)1(2a)(beta or b)(w)catenin?
L109         12 FILE MEDLINE
L110         17 FILE CAPLUS
L111         12 FILE BIOSIS
L112         11 FILE EMBASE
L113          2 FILE WPIDS

TOTAL FOR ALL FILES
L114         54 LEF(W) 1(2A)(BETA OR B)(W) CATENIN?

=> dup rem l114
PROCESSING COMPLETED FOR L114
L115         19 DUP REM L114 (35 DUPLICATES REMOVED)

=> d cbib abs 1-19;s author m?/au,in;s mukesh j?/au,in;s masafumi w?/au,in

L115 ANSWER 1 OF 19  MEDLINE                              DUPLICATE 1
2000261515  Document Number: 20261515.  Induction of melanocyte-specific
     microphthalmia-associated transcription factor by Wnt-3a. Takeda K;
     Yasumoto K; Takada R; Takada S; Watanabe K; Udono T; Saito H; Takahashi K;
     Shibahara S. (Department of Molecular Biology and Applied Physiology,
     Tohoku University School of Medicine, Aoba-ku, Sendai, Miyagi 980-8575,
```

Japan.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 May 12) 275 (19) 14013-6. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language:
English.
AB Microphthalmia-associated transcription factor (Mitf) plays a critical role in the development of neural crest-derived melanocytes. Here, we show
that exogenously added Wnt-3a protein, an intercellular signaling molecule, up-regulates the expression of endogenous melanocyte-specific Mitf (Mitf-M) mRNA in cultured melanocytes. The melanocyte-specific promoter of the human MITF gene (MITF-M promoter) contains a functional LEF-1-binding site, which is bound in vitro by LEF-1 and confers the preferential expression on a reporter gene in melanocytes and melanoma cells, as judged by the transient transfection assays. Moreover, the LEF-1-binding site is required for the transactivation of a reporter gene by **LEF-1**, **beta-catenin**, or their
combination. Exogenously added Wnt-3a protein also transactivates the MITF-M promoter via the LEF-1-binding site; this activation was abolished when a dominant-negative form of LEF-1 was coexpressed. These results suggest that Wnt-3a signaling recruits beta-catenin and LEF-1 to the LEF-1-binding site of the MITF-M promoter. Therefore, the present study identifies Mitf-M/MITF-M as a direct target of Wnt signaling.

L115 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2
2000:142338 Brachyury is a target gene of the Wnt/.beta.-catenin signaling pathway. Arnold, S. J.; Stappert, J.; Bauer, A.; Kispert, A.; Herrmann, B. G.; Kemler, R. (Max-Planck Institut fur Immunbiologie, Freiburg, D-79108, Germany). Mech. Dev., 91(1,2), 249-258 (English) 2000. CODEN: MEDVE6. ISSN: 0925-4773. Publisher: Elsevier Science Ireland Ltd..
AB To identify target genes of the Wnt/.beta.-catenin signaling pathway in early mouse embryonic development we have established a co-culture system consisting of NIH3T3 fibroblasts expressing different Wnts as feeder layer
cells and embryonic stem (ES) cells expressing a green fluorescent protein
(GFP) reporter gene transcriptionally regulated by the TCF/.beta.-catenin complex. ES cells specifically respond to Wnt signal as monitored by GFP expression. In GFP-pos. ES cells we observe expression of Brachyury. Two
TCF binding sites located in a 500 bp Brachyury promoter fragment bind the
LEF-1/.beta.-catenin complex and
respond specifically to .beta.-catenin-dependent transactivation. From these results we conclude that Brachyury is a target gene for Wnt/.beta.-catenin signaling.

L115 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3
1999:299499 Document No. 130:322697 An in vitro system for inducing neural crest cell differentiation to vascular smooth muscle cells and its use in identifying regulators and their genes. (President and Fellows of Harvard College, USA; Lee, Arthur M. E.; Jain, Mukesh; Watanabe, Masafumi). PCT Int. Appl. WO 9921965 A2 19990506, 92 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US22897 19981028. PRIORITY: US 1997-63363 19971028; US 1998-80420 19980402; US 1998-96685 19980814.

AB This invention is directed to an in vitro system for rapidly and uniformly inducing immortalized neural crest cells to differentiate to vascular smooth muscle cells. As excessive proliferation of vascular smooth muscle cells is a phenotypic response to the development of occlusive arteriosclerotic disease, the in vitro system of this invention is used to identify mol. regulators of smooth muscle cell development and differentiation. As the mol. regulators of smooth muscle cell differentiation are identified, the invention also encompasses methods to isolate the genes coding for these regulators. This invention also relates to mols. identified through the use of the invention's in vitro system, as well as to compds. that inhibit or regulate the identified mols. Neural crest Monc-1 cells were induced to differentiate to vascular smooth muscle cells by application of a smooth muscle cell differentiation medium (SMDM) contg. inorg. salts, amino acids, vitamins, and other components (specific components are listed) supplemented with 10% fetal bovine serum, penicillin, streptomycin and Hepes (pH 7.4).

L115 ANSWER 4 OF 19 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 2000-011873 [01] WPIDS
AB US 5949228 A UPAB: 20000524
NOVELTY - The feedback circuit includes a sensory circuit (110) that continually senses variations in process, temperature and power supply. The sensor information is provided to the top current mirror circuit (105) which adjusts its parameters accordingly.
USE - Feedback circuit for an integrated circuit.
ADVANTAGE - The feedback circuit improves the accuracy of the integrated circuit by compensating for process, temperature and power supply variations.
DESCRIPTION OF DRAWING(S) - The drawing shows a circuit diagram of the feedback circuit.
Top mirror circuit 105
Sensory circuit 110
Dwg.2/3

L115 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2000 ACS
1999:408642 Document No. 131:181426 Functional characterization of multiple transactivating elements in .beta.-catenin, some of which interact with the TATA-binding protein in vitro. Hecht, Andreas; Litterst, Claudia M.; Huber, Otmar; Kemler, Rolf (Max-Planck-Institute of Immunobiology, Freiburg, D-79108, Germany). J. Biol. Chem., 274(25), 18017-18025 (English) 1999. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB .beta.-Catenin, a member of the family of Armadillo repeat proteins, plays a dual role in cadherin-mediated cell adhesion and in signaling by Wnt growth factors. Upon Wnt stimulation .beta.-catenin undergoes nuclear translocation and serves as transcriptional coactivator of T cell factor DNA-binding proteins. Previously the transactivation potential of different portions of .beta.-catenin has been demonstrated, but the precise location of transactivating elements has not been established. Also, the mechanism of transactivation by .beta.-catenin and the mol. basis for functional differences between .beta.-catenin and the closely related proteins Armadillo and Plakoglobin are poorly understood. Here the authors have used a yeast system for the detailed characterization of the transactivation properties of .beta.-catenin. The authors show that

its transactivation domains possess a modular structure, consist of multiple subelements that cover broad regions at its N and C termini, and extend considerably into the Armadillo repeat region. Compared with .beta.-catenin the N termini of Plakoglobin and Armadillo have different transactivation capacities that may explain their distinct signaling properties. Furthermore, transactivating elements of .beta.-catenin interact specifically and directly with the TATA-binding protein in vitro providing further evidence that a major function of .beta.-catenin during Wnt signaling is to recruit the basal transcription machinery to promoter regions of Wnt target genes.

L115 ANSWER 6 OF 19 MEDLINE DUPLICATE 4
1999254073 Document Number: 99254073. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Shtutman M; Zhurinsky J; Simcha I; Albanese C; D'Amico M; Pestell R; Ben-Ze'ev A. (Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 May 11) 96 (10) 5522-7. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB beta-Catenin plays a dual role in the cell: one in linking the cytoplasmic side of cadherin-mediated cell-cell contacts to the actin cytoskeleton and an additional role in signaling that involves transactivation in complex with transcription factors of the lymphoid enhancing factor (**LEF -1**) family. Elevated **beta-catenin** levels in colorectal cancer caused by mutations in beta-catenin or by the adenomatous polyposis coli molecule, which regulates beta-catenin degradation, result in the binding of beta-catenin to LEF-1 and increased transcriptional activation of mostly unknown target genes. Here, we show that the cyclin D1 gene is a direct target for transactivation by the beta-catenin/LEF-1 pathway through a LEF-1 binding site in the cyclin D1 promoter. Inhibitors of beta-catenin activation, wild-type adenomatous polyposis coli, axin, and the cytoplasmic tail of cadherin suppressed cyclin D1 promoter activity in colon cancer cells. Cyclin D1 protein levels were induced by beta-catenin overexpression and reduced in cells overexpressing the cadherin cytoplasmic domain. Increased beta-catenin levels may thus promote neoplastic conversion by triggering cyclin D1 gene expression and, consequently, uncontrolled progression into the cell cycle.

L115 ANSWER 7 OF 19 MEDLINE DUPLICATE 5
1999263020 Document Number: 99263020. Nuclear localization and formation of beta-catenin-lymphoid enhancer factor 1 complexes are not sufficient for activation of gene expression. Prieve M G; Waterman M L. (Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, Irvine, California 92697-4025, USA.) MOLECULAR AND CELLULAR BIOLOGY, (1999 Jun) 19 (6) 4503-15. Journal code: NGY. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB In response to activation of the Wnt signaling pathway, beta-catenin accumulates in the nucleus, where it cooperates with LEF/TCF (for lymphoid enhancer factor and T-cell factor) transcription factors to activate gene expression. The mechanisms by which beta-catenin undergoes this shift in location and participates in activation of gene transcription are unknown. We demonstrate here that beta-catenin can be imported into the nucleus

independently of LEF/TCF binding, and it may also be exported from nuclei.
We have introduced a small deletion within beta-catenin (Delta19) that disrupts binding to LEF-1, E-cadherin, and APC but not axin. This Delta19 beta-catenin mutant localizes to the nucleus because it may not be efficiently sequestered in the cytoplasm. The nuclear localization of Delta19 definitively demonstrates that the mechanisms by which beta-catenin localizes in the nucleus are completely independent of LEF/TCF factors. beta-Catenin and LEF-1 complexes can activate reporter gene expression in a transformed T-lymphocyte cell line (Jurkat) but not in normal T lymphocytes, even though both factors are nuclear. Thus, localization of both factors to the nucleus is not sufficient for activation of gene expression. Excess beta-catenin can squelch reporter gene activation by **LEF-1-beta-catenin** complexes but not activation by the transcription factor VP16. Taken together, these data suggest that a third component is necessary for gene activation and that this third component may vary with cell type.

L115 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2000 ACS
2000:401232 Mechanism of chromatin recognition and transcriptional regulation by LEF-1 and the Wnt/Wg-responsive **LEF-1:.beta.-catenin** complex. Tutter, A.; McAlpine, G. S.; Jones, K. A. (Regulatory Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, 92037-1099, USA). Cold Spring Harbor Symp. Quant. Biol., 64(Signaling and Gene Expression in the Immune System), 445-452 (English) 1999. CODEN: CSHSAZ. ISSN: 0091-7451. Publisher: Cold Spring Harbor Laboratory Press.
AB Unavailable

L115 ANSWER 9 OF 19 MEDLINE DUPLICATE 6
1999093503 Document Number: 99093503. Nuclear endpoint of Wnt signaling: neoplastic transformation induced by transactivating lymphoid-enhancing factor 1. Aoki M; Hecht A; Kruse U; Kemler R; Vogt P K. (Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, BCC-239, La Jolla, CA 92037, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Jan 5) 96 (1) 139-44. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB The interaction between beta-catenin and LEF-1/TCF transcription factors plays a pivotal role in the Wnt-1 signaling pathway. The level of beta-catenin is regulated by partner proteins, including glycogen synthase kinase-3beta (GSK-3beta) and the adenomatous polyposis coli (APC) tumor suppressor protein. Genetic defects in APC are responsible for a heritable predisposition to colon cancer. APC protein and GSK-3beta bind beta-catenin, retain it in the cytoplasm, and facilitate the proteolytic degradation of beta-catenin. Abrogation of this negative regulation allows beta-catenin to translocate to the nucleus and to form a transcriptional activator complex with the DNA-binding protein lymphoid-enhancing factor 1 (LEF-1). This complex is thought to be involved in tumorigenesis. Here we show that covalent linkage of **LEF-1** to **beta-catenin** and to transcriptional activation domains derived from the estrogen receptor or the herpes simplex virus protein VP16 generates transcriptional regulators that induce oncogenic transformation of chicken

embryo fibroblasts. The chimeras between **LEF-1** and **beta-catenin** or VP16 are constitutively active, whereas fusions of LEF-1 to the estrogen receptor are regulatable by estrogen. These experiments document the oncogenicity of transactivating LEF-1 and show that the transactivation domain normally provided by beta-catenin can be replaced by heterologous activation domains. These results suggest that the transactivating function of the **LEF-1/beta-catenin** complex is critical for tumorigenesis and that this complex transforms cells by activating specific LEF-1 target genes.

L115 ANSWER 10 OF 19 MEDLINE DUPLICATE 7
1999264277 Document Number: 99264277. The C-terminal transactivation domain of beta-catenin is necessary and sufficient for signaling by the **LEF-1/beta-catenin** complex in Xenopus laevis. Vleminckx K; Kemler R; Hecht A. (Max-Planck-Institute of Immunobiology, Stuebeweg 51, D-79108, Freiburg, Germany.) MECHANISMS OF DEVELOPMENT, (1999 Mar) 81 (1-2) 65-74. Journal code: AXF. ISSN: 0925-4773. Pub. country: Ireland. Language: English.

AB Beta-catenin is a multifunctional protein involved in cell adhesion and communication. In response to signaling by Wnt growth factors, beta-catenin associates with nuclear TCF factors to activate target genes.

A transactivation domain identified at the C-terminus of beta-catenin can stimulate expression of artificial reporter genes. However, the mechanism of target gene activation by TCF/beta-catenin complexes and the physiological relevance of the beta-catenin transactivation domain still remain unclear. Here we asked whether the beta-catenin transactivation domain can generate a Wnt-response in a complex biological system, namely axis formation during Xenopus laevis embryogenesis. We show that a chimeric transcription factor consisting of beta-catenin fused to the DNA-binding domain of LEF-1 induces a complete secondary dorsoanterior axis when expressed in Xenopus. A **LEF-1-beta-catenin** fusion lacking the C-terminal transactivation domain is impaired in signaling while fusion of just the beta-catenin transactivator to the DNA-binding domain of LEF-1 is sufficient for axis-induction. The latter fusion molecule is blocked by dominant negative LEF-1 but not by excess cadherin indicating that all events parallel or upstream of the transactivation step mediated by beta-catenin are dispensable for Wnt-signaling. Moreover, beta-catenin can be replaced by a heterologous transactivator. Apparently, the ultimate function of beta-catenin in Wnt signaling is to recruit the basal transcription machinery to promoter regions of specific target genes.

L115 ANSWER 11 OF 19 MEDLINE DUPLICATE 8
2000131853 Document Number: 20131853. The dual role of cytoskeletal anchor proteins in cell adhesion and signal transduction. Ben-Ze'ev A. (Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel.. Lgbenzev@weizmann.weizmann.ac.il) . ANNALS OF THE NEW YORK ACADEMY OF SCIENCES,. (1999) 886 37-47. Ref: 41. Journal code: 5NM. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB beta-Catenin and plakoglobin are homologous proteins having a dual role in cell adhesion and in transactivation together with LEF/TCF transcription factors. Overexpression of plakoglobin suppresses tumorigenicity, whereas increased beta-catenin levels are considered oncogenic. We compared the nuclear translocation and transactivation by beta-catenin and plakoglobin.

Overexpression of each protein resulted in nuclear translocation and

formation of structures that also contained LEF-1 and vinculin with beta-catenin, but not with plakoglobin. Transfection of **LEF-1** translocated endogenous **beta-catenin**, but not plakoglobin into the nucleus. Chimeras of the Gal4 DNA-binding domain and the transactivation domains of either plakoglobin or beta-catenin were equally potent in transactivation, but induction of LEF-1-responsive transcription was higher with beta-catenin. Overexpression of wt plakoglobin or mutant beta-catenin lacking the transactivation domain induced nuclear accumulation of the endogenous beta-catenin and LEF-1-responsive transactivation. The nuclear localization and constitutive beta-catenin-dependent transactivation in SW480 cancer cells were inhibited by overexpressing cadherin or alpha-catenin. Moreover, transfecting the cytoplasmic tail of cadherin inhibited transactivation, by competition with LEF-1 in the nucleus for beta-catenin binding. The results indicate that (1) plakoglobin and beta-catenin differ in nuclear translocation and complexing with LEF-1 and vinculin, (2) LEF-1-dependent transactivation is mainly driven by beta-catenin, (3) cadherin and alpha-catenin can sequester beta-catenin, inhibit its transcriptional activity, and antagonize its oncogenic action.

L115 ANSWER 12 OF 19 MEDLINE DUPLICATE 9
1998426198 Document Number: 98426198. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. Levanon D; Goldstein R E; Bernstein Y; Tang H; Goldenberg D; Stifani S; Paroush Z; Groner Y. (Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Sep 29) 95 (20) 11590-5. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The mammalian AML/CBFalpha runt domain (RD) transcription factors regulate hematopoiesis and osteoblast differentiation. Like their Drosophila counterparts, most mammalian RD proteins terminate in a common pentapeptide, VWRPY, which serves to recruit the corepressor Groucho (Gro). Using a yeast two-hybrid assay, in vitro association and pull-down experiments, we demonstrate that Gro and its mammalian homolog TLE1 specifically interact with AML1 and AML2. In addition to the VWRPY motif, other C-terminal sequences are required for these interactions with Gro/TLE1. TLE1 inhibits AML1-dependent transactivation of the T cell receptor (TCR) enhancers alpha and beta, which contain functional AML binding sites, in transfected Jurkat T cells. LEF-1 is an additional transcription factor that mediates transactivation of TCR enhancers. LEF-1 and its Drosophila homolog Pangolin (Pan) are involved in the Wnt/Wg signaling pathway through interactions with the coactivator beta-catenin and its highly conserved fly homolog Armadillo (Arm). We show that TLE/Gro interacts with LEF-1 and Pan, and inhibits **LEF-1**: **beta-catenin**-dependent transcription. These data indicate that, in addition to their activity as transcriptional activators, AML1 and LEF-1 can act, through recruitment of the corepressor TLE1, as transcriptional repressors in TCR regulation and Wnt/Wg signaling.

L115 ANSWER 13 OF 19 MEDLINE DUPLICATE 10
1998208547 Document Number: 98208547. Cell adhesion and the integrin-linked kinase regulate the **LEF-1** and **beta-catenin** signaling pathways. Novak A; Hsu S C; Leung-Hagesteijn C;

Radeva G; Papkoff J; Montesano R; Roskelley C; Grosschedl R; Dedhar S. (Division of Cancer Research, Sunnybrook Health Science Centre, Research Building, S-218, 2075 Bayview Avenue, Toronto, Ontario, Canada M4N 3M5.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Apr 14) 95 (8) 4374-9. Journal code: PV3. ISSN: 0027-8424.

Pub. country: United States. Language: English.

AB The integrin-linked kinase (ILK) is an ankyrin repeat containing serine-threonine protein kinase that can interact directly with the cytoplasmic domains of the beta1 and beta3 integrin subunits and whose kinase activity is modulated by cell-extracellular matrix interactions. Overexpression of constitutively active ILK results in loss of cell-cell adhesion, anchorage-independent growth, and tumorigenicity in nude mice. We now show that modest overexpression of ILK in intestinal epithelial cells as well as in mammary epithelial cells results in an invasive phenotype concomitant with a down-regulation of E-cadherin expression, translocation of beta-catenin to the nucleus, formation of a complex between beta-catenin and the high mobility group transcription factor, LEF-1, and transcriptional activation by this **LEF-1/beta-catenin** complex. We also find that LEF-1 protein expression is rapidly modulated by cell detachment from the extracellular matrix, and that LEF-1 protein levels are constitutively up-regulated at ILK overexpression. These effects are specific for ILK, because transformation by activated H-ras or v-src oncogenes do not result in the activation of **LEF-1/beta-catenin**. The results demonstrate that the oncogenic properties of ILK involve activation of the **LEF-1/beta-catenin** signaling pathway, and also suggest ILK-mediated cross-talk between cell-matrix interactions and cell-cell adhesion as well as components of the Wnt signaling pathway.

L115 ANSWER 14 OF 19 MEDLINE DUPLICATE 11

1998292519 Document Number: 98292519. Differential nuclear translocation and transactivation potential of beta-catenin and plakoglobin. Simcha I; Shtutman M; Salomon D; Zhurinsky J; Sadot E; Geiger B; Ben-Ze'ev A. (Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel.) JOURNAL OF CELL BIOLOGY, (1998 Jun 15) 141 (6) 1433-48. Journal code: HMV. ISSN: 0021-9525. Pub. country: United States.

Language: English.

AB beta-Catenin and plakoglobin are homologous proteins that function in cell adhesion by linking cadherins to the cytoskeleton and in signaling by transactivation together with lymphoid-enhancing binding/T cell (LEF/TCF) transcription factors. Here we compared the nuclear translocation and transactivation abilities of beta-catenin and plakoglobin in mammalian cells. Overexpression of each of the two proteins in MDCK cells resulted in nuclear translocation and formation of nuclear aggregates. The beta-catenin-containing nuclear structures also contained LEF-1 and vinculin, while plakoglobin was inefficient in recruiting these molecules, suggesting that its interaction with LEF-1 and vinculin is significantly weaker. Moreover, transfection of **LEF-1** translocated endogenous **beta-catenin**, but not plakoglobin to the nucleus. Chimeras consisting of Gal4 DNA-binding domain and the transactivation domains of either plakoglobin or beta-catenin were equally potent in transactivating a Gal4-responsive reporter, whereas activation of LEF-1- responsive transcription was significantly higher with beta-catenin. Overexpression of wild-type plakoglobin or mutant

beta-catenin lacking the transactivation domain induced accumulation of the endogenous beta-catenin in the nucleus and LEF-1-responsive transactivation. It is further shown that the constitutive beta-catenin-dependent transactivation in SW480 colon carcinoma cells and its nuclear localization can be inhibited by overexpressing N-cadherin or alpha-catenin. The results indicate that (a) plakoglobin and beta-catenin differ in their nuclear translocation and complexing with LEF-1 and vinculin; (b) LEF-1-dependent transactivation is preferentially driven by beta-catenin; and (c) the cytoplasmic partners of beta-catenin, cadherin and alpha-catenin, can sequester it to the cytoplasm and inhibit its transcriptional activity.

L115 ANSWER 15 OF 19 MEDLINE DUPLICATE 12
1998080141 Document Number: 98080141. Induction of a beta-catenin-LEF-1 complex by wnt-1 and transforming mutants of beta-catenin. Porfiri E; Rubinfeld B; Albert I; Hovanes K; Waterman M; Polakis P. (ONYX Pharmaceuticals, Richmond, California 94806, USA.) ONCOGENE, (1997 Dec 4)
15 (23) 2833-9. Journal code: ONC. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Signal transduction by beta-catenin involves its posttranslational stabilization and import to the nucleus where it interacts with transcription factors. Recent implications for beta-catenin signaling in cancer prompted us to examine colon cancer cell lines for the expression of LEF-1, a transcription factor that binds to beta-catenin. The analysis of several cell lines revealed the expression of LEF1 mRNA and a constitutive association of the **LEF-1** protein with **beta-catenin**. In contrast to the colon cells, PC12 and 293 cells did not contain a beta-catenin-LEF-1 complex, even though both proteins were detected in cell lysates. In these cells, the association of endogenous LEF1 and beta-catenin was induced by stimulation with the wnt-1 proto-oncogene. The complex formed following transient stimulation with wnt-1 and also persisted in cells stably expressing wnt-1. Ectopic overexpression of beta-catenin in 293 cells also induced the assembly of the beta-catenin-LEF-1 complex and activated gene transcription from a LEF-1-dependent promotor. Expression of mutant oncogenic forms of beta-catenin identified in cancer cells resulted in higher levels of transcriptional activity. The results suggest that a cancer pathway driven by wnt-1, or mutant forms of beta-catenin, may involve the formation of a persistent transcriptionally active complex of beta-catenin and LEF1.

L115 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2000 ACS
1997:205878 Document No. 126:304371 Stabilization of .beta.-catenin by genetic defects in melanoma cell lines. Rubinfeld, Bonnee; Robbins, Paul; El-Gamil, Mona; Albert, Iris; Porfiri, Emilio; Polakis, Paul (Onyx Pharmaceuticals, Richmond, CA, 94806, USA). Science (Washington, D. C.), 275(5307), 1790-1792 (English) 1997. CODEN: SCIEAS. ISSN: 0036-8075. Publisher: American Association for the Advancement of Science.
AB Signal transduction by .beta.-catenin involves its posttranslational stabilization and downstream coupling to the Lef and Tcf transcription factors. Abnormally high amts. of .beta.-catenin were detected in 7 of 26 human melanoma cell lines. Unusual mRNA splicing and missense mutations in the .beta.-catenin gene (CTNNB1) that result in stabilization of the protein were identified in six of the lines, and the adenomatous polyposis coli tumor suppressor protein (APC) was altered or missing in two others.

In the APC-deficient cells, ectopic expression of wild-type APC eliminated the excess .beta.-catenin. Cells with stabilized .beta.-catenin contained a constitutive .beta.-catenin-Lef-1 complex. Thus, genetic defects that result in up-regulation of .beta.-catenin may play a role in melanoma progression.

L115 ANSWER 17 OF 19 BIOSIS COPYRIGHT 2000 BIOSIS
1998:20218 Document No.: PREV199800020218. The oncogene integrin linked kinase (ILK) activates the **LEF-1/beta-catenin** signaling pathway. Novak, A. (1); Hsu, S.-C.; Leung-Hagesteijn, C. (1); Radeva, G. (1); Papkoff, J.; Montesano, R.; Grossched, R.; Dedhar, S. (1). (1) Sunnybrook Health Sci. Cent., Toronto, ON M4N 3M5 Canada. Molecular Biology of the Cell, (Nov., 1997) Vol. 8, No. SUPPL., pp. 136A. Meeting Info.: 37th Annual Meeting of the American Society for Cell Biology Washington, D.C., USA December 13-17, 1997 American Society for Cell Biology. ISSN: 1059-1524. Language: English.

L115 ANSWER 18 OF 19 MEDLINE DUPLICATE 13
96338228 Document Number: 96338228. Functional interaction of beta-catenin with the transcription factor LEF-1. Behrens J; von Kries J P; Kuhl M; Bruhn L; Wedlich D; Grosschedl R; Birchmeier W. (Max Delbruck Centre for Molecular Medicine, Berlin, Germany.) NATURE, (1996 Aug 15) 382 (6592) 638-42. Journal code: NSC. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The cytoplasmic proteins beta-catenin of vertebrates and armadillo of Drosophila have two functions: they link the cadherin cell-adhesion molecules to the cytoskeleton, and they participate in the wnt/wingless signal pathway. Here we show, in a yeast two-hybrid screen, that the architectural transcription factor LEF-1 (for lymphoid enhancer-binding factor) interacts with beta-catenin. In mammalian cells, coexpressed **LEF-1** and **beta-catenin** form a complex that is localized to the nucleus and can be detected by immunoprecipitation. Moreover, **LEF-1** and **beta-catenin** form a ternary complex with DNA that splays an altered DNA bend. Microinjection of LEF-1 into XenoPus embryos induces axis duplication, which is augmented by interaction with beta-catenin. Thus beta-catenin regulates gene expression by direct interaction with transcription factors such as LEF-1, providing a molecular mechanism for the transmission of signals, from cell-adhesion components or wnt protein to the nucleus.

L115 ANSWER 19 OF 19 MEDLINE DUPLICATE 14
97047308 Document Number: 97047308. Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. Huber O; Korn R; McLaughlin J; Ohsugi M; Herrmann B G; Kemler R. (Department of Molecular Embryology, Max-Planck Institute for Immunobiology, Freiburg, Germany.) MECHANISMS OF DEVELOPMENT, (1996 Sep) 59 (1) 3-10. Journal code: AXF. ISSN: 0925-4773. Pub. country: Ireland. Language: English.
AB Vertebrate beta-catenin and Drosophila Armadillo share structural similarities suggesting that beta-catenin, like Armadillo, has a developmental signaling function. Both proteins are present as components of cell adherens junctions, but accumulate in the cytoplasm upon Wingless/Wnt signaling. beta-Catenin has axis-inducing properties like Wnt when injected into Xenopus blastomeres, providing evidence for

participation of beta-catenin in the Wnt-pathway, but until now no downstream targets for beta-catenin have been identified. Here we demonstrate that beta-catenin binds to the HMG-type transcription factor lymphoid enhancer factor-1 (LEF-1), resulting in a nuclear translocation of beta-catenin both in cultured mouse cells and after ectopic expression of LEF-1 in two-cell mouse embryos. **LEF-1/beta -catenin** complexes bind to the promoter region of the E-cadherin gene in vitro, suggesting that this interaction could regulate E-cadherin transcription. As shown for beta-catenin, ectopic expression of LEF-1 in Xenopus embryos caused duplication of the body axis, indicating a regulatory role for a LEF-1-like molecule in dorsal mesoderm formation.

```
'IN' IS NOT A VALID FIELD CODE
L116          0 FILE MEDLINE
L117          0 FILE CAPLUS
L118          0 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L119          0 FILE EMBASE
L120          0 FILE WPIDS

TOTAL FOR ALL FILES
L121          0 AUTHOR M?/AU,IN


'IN' IS NOT A VALID FIELD CODE
L122          0 FILE MEDLINE
L123          0 FILE CAPLUS
L124          2 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L125          0 FILE EMBASE
L126          0 FILE WPIDS

TOTAL FOR ALL FILES
L127          2 MUKESH J?/AU,IN


'IN' IS NOT A VALID FIELD CODE
L128          0 FILE MEDLINE
L129          0 FILE CAPLUS
L130          0 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L131          0 FILE EMBASE
L132          0 FILE WPIDS

TOTAL FOR ALL FILES
L133          0 MASAFUMI W?/AU,IN

=> s watanabe m?/au,in
'IN' IS NOT A VALID FIELD CODE
L134       3224 FILE MEDLINE
L135       8809 FILE CAPLUS
L136       4867 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L137       2966 FILE EMBASE
L138       1359 FILE WPIDS

TOTAL FOR ALL FILES
L139      21225 WATANABE M?/AU,IN
```

=> s l49 and l139
L140 1 FILE MEDLINE
L141 1 FILE CAPLUS
L142 2 FILE BIOSIS
L143 1 FILE EMBASE
L144 0 FILE WPIDS

TOTAL FOR ALL FILES
L145 5 L49 AND L139

=> dup rem l145
PROCESSING COMPLETED FOR L145
L146 2 DUP REM L145 (3 DUPLICATES REMOVED)

=> d cbib abs 1-2

L146 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
1998165765 Document Number: 98165765. In vitro system for **differentiating** pluripotent **neural crest** cells into **smooth muscle** cells. Jain M K; Layne M D; **Watanabe M**; Chin M T; Feinberg M W; Sibinga N E; Hsieh C M; Yet S F; Stemple D L; Lee M E. (Cardiovascular Biology Laboratory, Harvard School of Public Health, Boston, Massachusetts 02115, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 13) 273 (11) 5993-6. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The change in vascular **smooth muscle** cells (SMC) from a **differentiated** to a dedifferentiated state is the critical phenotypic response that promotes occlusive arteriosclerotic disease. Despite its importance, research into molecular mechanisms regulating **smooth muscle differentiation** has been hindered by the lack of an in vitro cell **differentiation** system. We identified culture conditions that promote efficient **differentiation** of Monc-1 pluripotent **neural crest** cells into SMC. Exclusive Monc-1 to SMC **differentiation** was indicated by cellular morphology and time-dependent induction of the SMC markers **smooth muscle** alpha-actin, **smooth muscle** myosin heavy chain, calponin, SM22alpha, and APEG-1. The activity of the SM22alpha promoter was low in Monc-1 cells. **Differentiation** of these cells into SMC caused a 20-30-fold increase in the activity of the wild-type SM22alpha promoter and that of a hybrid promoter containing three copies of the CArG element. By gel mobility shift analysis, we identified new DNA-protein complexes in nuclear extracts prepared from **differentiated** Monc-1 cells. One of the new complexes contained serum response factor. This Monc-1 to SMC model should facilitate the identification of nodal regulators of **smooth muscle** development and **differentiation**.

L146 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2000 BIOSIS
1999:524403 Document No.: PREV199900524403. Identification of latent transforming growth factor-beta binding protein as a gene markedly induced during **neural crest** cell to **smooth muscle** cell **differentiation**. **Watanabe, Masafumi** ; Layne, Matthew D.; Jain, Mukesh K.; Hsieh, Chung-Ming; Chin, Michael T.; Yet, Shaw-Fang; Lee, Mu-En. Harvard Sch. Public Health, Boston, MA USA. Circulation, (Oct. 27, 1998) Vol. 98, No. 17 SUPPL., pp. I605. Meeting Info.: 71st Scientific Sessions of the American Heart Association Dallas, Texas, USA November 8-11, 1998 The American Heart Association. ISSN: 0009-7322. Language: English.

AN 1999:163509 USPATFULL
TI Methods for differentiating neural stem cells to neurons or smooth muscle cells using TGT-.beta. super family growth factors
IN Anderson, David J., Altadena, CA, United States
Shah, Nirao M., New York, NY, United States
PA California Institute of Technology, Pasadena, CA, United States (U.S. corporation)
PI US 6001654 19991214
AI US 1997-846028 19970425 (8)
RLI Continuation-in-part of Ser. No. US 1994-188286, filed on 28 Jan 1994, now patented, Pat. No. US 5654183 which is a continuation-in-part of Ser. No. WO 1993-US7000, filed on 26 Jul 1993 which is a continuation-in-part of Ser. No. US 1992-969088, filed on 29 Oct 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-920617, filed on 27 Jul 1992, now abandoned
PRAI US 1997-44797 19970424 (60)
DT Utility
EXNAM Primary Examiner: LaGuyader, John L.; Assistant Examiner: Shibuya, Mark L.
LREP Mintz, Levin, Cohn, Ferris, Glovsky and Popeo P.C.; Elrifi, Ivor R.; Primce, John T.
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 25 Drawing Figure(s); 28 Drawing Page(s)
LN.CNT 2392
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Method for producing a population of mammalian neurons and/or smooth muscle cells comprising contacting at least one mammalian neural stem cell with a culture medium containing one or more growth factors from the TGF-.beta. super family and detecting the differentiation of stem cell to a population of neurons or smooth muscle cells.

102(e)

AN 97:88884 USPATFULL
TI Immoralized neural crest stem cells and methods of making
IN Anderson, David J., Altadena, CA, United States
Stemple, Derek L., Newton, MA, United States
PA California Institute of Technology, Pasadena, CA, United States (U.S. corporation)
PI US 5672499 19970930
AI US 1995-478920 19950607 (8)
RLI Division of Ser. No. US 1994-188286, filed on 28 Jan 1994 which is a continuation-in-part of Ser. No. US 1992-969088, filed on 29 Oct 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-920617, filed on 27 Jul 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Leguyader, John L.
LREP Flehr Hohbach Test Albritton Herbert LLP; Trecartin, Richard F.; Silva, Robin M.
CLMN Number of Claims: 8
ECL Exemplary Claim: 1,2
DRWN 62 Drawing Figure(s); 23 Drawing Page(s)
LN.CNT 2112
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention includes mammalian multipotent neural stem cells and their

progeny and methods for the isolation and clonal propagation of such cells. At the clonal level the stem cells are capable of self regeneration and asymmetrical division. Lineage restriction is demonstrated within developing clones which are sensitive to the local environment. The invention also includes such cells which are transfected with foreign nucleic acid, e.g., to produce an immortalized neural stem cell. The invention further includes transplantation assays which allow for the identification of mammalian multipotent neural stem cells from various tissues and methods for transplanting mammalian neural stem cells and/or neural or glial progenitors into mammals. A novel method for detecting antibodies to neural cell surface markers is disclosed as well as a monoclonal antibody to mouse LNGFR.

09/181,311

L7 ANSWER 1 OF 2 USPATFULL
AN 1999:78606 USPATFULL
TI High efficiency gene trap selection of regulated genetic loci
IN Baetscher, Manfred, Winchester, MA, United States
Nir, Waan-Jeng, Reading, MA, United States
PA BioTransplant, Inc., Charlestown, MA, United States (U.S. corporation)
PI US 5922601 19990713
AI US 1996-716854 19960916 (8)
RLI Continuation of Ser. No. US 1995-374833, filed on 19 Jan 1995, now abandoned
DT Utility
EXNAM Primary Examiner: Railey, II, Johnny F.
LREP Olstein, Elliot M.; Semionow, Raina
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 1637
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A gene trap construct for identification of genes whose activity is regulated upon a cellular transition event which comprises in downstream sequence (i) a cassette having a functional splice acceptor, a translation stop sequence and an internal ribosome entry site and (ii) a promoterless protein coding sequence encoding at least one polypeptide providing positive and negative selection traits. A method for identification of genes whose activity is regulated upon a cellular transition event by introducing the gene trap construct into a cell and observing expression of the positive and/or negative selection traits before and after the transition event.

L7 ANSWER 2 OF 2 USPATFULL
AN 1998:143938 USPATFULL
TI Neuronal-neonatal gene: neuronatin
IN Joseph, Rajiv, Birmingham, MI, United States
Dou, Dexian, Dearborn, MI, United States
PA Henry Ford Health System, Detroit, MI, United States (U.S. corporation)
PI US 5837535 19981117
AI US 1996-602093 19960215 (8)
RLI Continuation-in-part of Ser. No. US 1994-259299, filed on 13 Jun 1994, now abandoned
DT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Hayes, Robert C.
LREP Kohn & Associates
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 2726
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is an isolated and purified DNA sequence which encodes a vertebrate mRNA for a neuron specific protein, neuronatin. The mRNA is selectively expressed in brain tissue during rapid brain growth when there is a surge in neuronal proliferation and migration and is repressed in adult tissue. In the human, the genomic DNA is as set forth in SEQ ID No:6 and the cDNA has a nucleotide sequence as set forth in SEQ ID No:5, with the gene mapped to human chromosome 20q11.2-12. The deduced protein is a proteolipid that appears to have a role in ion

channel regulation during brain development.

=> d kwic 1-

YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L7 ANSWER 1 OF 2 USPATFULL

SUMM . . . the subtraction. This approach has been employed with various degrees of success. Another suitable approach is PCR based and termed " **differential display**" (Liang and Pardee 1992; Liang et al 1993) It uses subsets of random PCR primers to amplify unique messages. This. . .

DRWD A family of master regulatory genes was identified and is being characterized in the cellular lineage of skeletal **muscle** differentiation. It consists of several members of DNA binding proteins termed MyoD (Thayer et al. 1989). Molecular analysis of **muscle** differentiation became feasible because of the availability of a tissue culture system that could reliably be induced to differentiate in vitro into skeletal **muscle**. The cell line 10T 1/2 can differentiate along various lineages and normally fits the characteristic of a fibroblast. However, when exposed to certain culture conditions, it differentiates into skeletal **muscle** or adipocytes. Induction of differentiation is achieved upon culture of these cells in 5-azacytidine. The replicating DNA becomes hypomethylated which. . . 10T1/2, that apparently is affected in its level of expression by hypomethylation is the MyoD master regulatory gene, which causes **muscle** differentiation.

DRWD Cell types that cause particularly devastating forms of tumors include some of the **neural crest** derived cells including the small cells of the lung, melanocytes, chromaffin cells in the adrenal medulla and parafollicular cells in. . .

L7 ANSWER 2 OF 2 USPATFULL

DRWD FIG. 1 is a photograph of a **differential display** gel of brain cDNA from healthy 3 day old (neonate (N)), 3 month old (young adult (Y)) and 33 month. . .

DRWD . . . brain RNA from neonatal (N), young adult (Y), and aged adult (A) rats using the cDNA fragment extracted from the **differential display** gel as the probe (FIG. 1) and ethidium bromide stained gels pictured under UV light prior to transfer showing that. . .

DETD The present invention was developed by extracting total RNA from neonatal, young adult and aged rat brain and processed for **differential display**. A cDNA fragment selectively expressed in neonatal brain was identified (FIG. 1). This cDNA fragment was extracted from the gel,. . .

DETD TABLE 2

THE PROTEOLIPID FAMILY OF AMPHIPATHIC POLYPETIDES

Amino Acids	Mol Wt (kDa)	Function	References
Sarcolipin			
31	3.8	Ca.sup.2+ -ATPase Skeletal **Muscle**	Wawrzynow et al 1992
PMP1			
40	4.7	H.sup.+ -ATPase Yeast	Navarre et al 1992
PMP2			
43	4.8	H.sup.+ -ATPase Yeast	Navarre et al 1994
Phospho-			
52	6.1	Ca.sup.2+ -ATPase	

lamban .gamma.-Subunit		Cardiac **Muscle**	Tada 1991
	58	6.5	Na.sup.+ /K.sup.+ - ATPase	Mercer 1993
Phospho-lemman	72	8.4	Choride Channel Taurine	Palmer et al 1991 Moorman et al

. . .

DETD . . . genes, SCG10.sup.95, sodium channel-II.sup.86 and synapsin-I.sup.88. SCG10 is growth-associated protein of 22kd that is expressed in neuronal derivatives of the **neural crest** . At E11.5 in the rat, it first becomes expressed in sympatho-adrenal progenitor cells. Thereafter, SCG10 levels are strongly upregulated in. . .

DETD **Differential Display**: Animals were decapitated, brain quickly dissected out, immersed in liquid nitrogen, homogenized and total RNA extracted by acid guanidinium thiocyanate-phenol-chloroform. . . ethanol precipitated and PCR amplified using the same set of primers and conditions used in the first amplification reaction for **differential display**. The presence of amplified PCR products was confirmed on 1.5% agarose gels.

DETD Northern Blotting: Initially, partial sequences extracted from the **differential display** gel were used as probes in Northern blots containing brain RNA from neonatal, young adult and aged rat brain. Once. . .

DETD Cloning and sequencing CDNA Fragments: cDNA fragment isolated from **differential display** gel were cloned into pCR.TM. (Invitrogen, San Diego, Calif.) as described..sup.8 The cloned vector was used to transform TA One. . .

DETD cDNA fragments isolated from a **differential display** gel were cloned.sup.8 and used to transform TA One Shot.TM. competent cells. White colonies were selected for plasmid preparation from. . .

DETD . . . brain of neonatal (3 days), young adult (3 months) and aged adult (33 months) rats, reverse transcribed into cDNA and **differentially displayed** on denaturing sequencing gels,.sup.37,42,68 as discussed herein above. A total of 35 cDNA bands which were selectively expressed in one. . .

DETD . . . respectively. In additional experiments using rat tissue, applicants noted that neuronatin mRNA was not expressed in the heart, skin and **muscle**.

DETD 111. Wawrzynow, et al, Sarcolipin, the "proteolipid" of skeletal **muscle** sarcoplasmic reticulum, is a unique, amphipathic, 31-residue peptide. Arch Biochem Biophys 298 (1992) 620-623.

L4 ANSWER 1 OF 2 USPATFULL
AN 2000:24750 USPATFULL
TI Conservin compositions
IN Falb, Dean A., Wellesley, MA, United States
Gimeno, Carlos J., Boston, MA, United States
PA Millenium Pharmaceuticals, Inc., Cambridge, MA, United States (U.S. corporation)
PI US 6031076 20000229
AI US 1998-2832 19980105 (9)
RLI Division of Ser. No. US 1996-688609, filed on 30 Jul 1996, now patented,
Pat. No. US 5807708
DT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Kaufman, Claire
M.
LREP Lahive & Cockfield, LLP; Mandragouras, Amy E.
CLMN Number of Claims: 52
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 3978
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the discovery of novel conservin genes and polypeptides. Therapeutics, diagnostics and screening assays based on these molecules are also disclosed.

=> d kwic 1-

YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 2 USPATFULL
SUMM . . . in the dorsal side of the developing chick neural tube (Basler et al. (1993)Cell 73:687-702). It promotes the outgrowth of **neural crest** cells and inhibits the formation of motor neuron cells in vitro, suggesting that it plays an important role in neural. . .
SUMM . . . Biol. 113:1439). The earliest lesion in atherosclerosis is the fatty streak, comprising lipid, macrophages, and T cells, which leads to **smooth muscle** cell proliferation and the development of more severe lesions (Ross. 1993 Nature 362:801). Transgenic mice which overexpress lipoprotein have been. . .
DETD . . . by their ability to induce or inhibit the proliferation of such cells as fibroblasts, cells of the immune system, or **smooth muscle** cells. Additional effects of conservin may be seen on tissue maintenance and repair, such as bone repair or wound healing..
.
DETD In preferred embodiments, a conservin protein can influence the proliferation of **smooth muscle** cells. In particularly preferred embodiments the subject polypeptides are capable of modulating the initiation and development of cardiovascular disease.
DETD . . . to various cancers and leukemias, psoriasis, bone diseases, fibroproliferative disorders such as those involving connective tissue, atherosclerosis, restenosis and other **smooth muscle** proliferative disorders, as well as chronic inflammation. In particular it is anticipated that mutation or deletion of both alleles of. . .

occur due to de-differentiation of chondrocytes or osteocytes, as well as vascular disorders which involve de-differentiation of endothelial tissue and **smooth muscle** cells, gastric ulcers characterized by degenerative changes in glandular cells, and renal conditions marked by failure to differentiate, e.g. Wilm's. . . .

DETD Any cell type or tissue, preferably monocytes, endothelial cells, or **smooth muscle** cells, in which the conservin is expressed may be utilized in the diagnostics described below. For example, a subject's bodily. . .

DETD . . . sequence, profiles may also be assessed in such detection schemes. Fingerprint profiles may be generated, for example, by utilizing a **differential display** procedure, Northern analysis and/or RT-PCR.

L4 ANSWER 2 OF 2 USPATFULL

SUMM . . . in the dorsal side of the developing chick neural tube (Basler et al. (1993)Cell 73:687-702). It promotes the outgrowth of **neural crest** cells and inhibits the formation of motor neuron cells in vitro, suggesting that it plays an important role in neural. . .

SUMM . . . Biol. 113:1439). The earliest lesion in atherosclerosis is the fatty streak, comprising lipid, macrophages, and T cells, which leads to **smooth muscle** cell proliferation and the development of more severe lesions (Ross. 1993 Nature 362:801). Transgenic mice which overexpress lipoprotein have been. . .

DETD . . . by their ability to induce or inhibit the proliferation of such cells as fibroblasts, cells of the immune system, or **smooth muscle** cells. Additional effects of conservin may be seen on tissue maintenance and repair, such as bone repair or wound healing..

.

DETD In preferred embodiments, a conservin protein can influence the proliferation of **smooth muscle** cells. In particularly preferred embodiments the subject polypeptides are capable of modulating the initiation and development of cardiovascular disease.

DETD . . . to various cancers and leukemias, psoriasis, bone diseases, fibroproliferative disorders such as those involving connective tissue, atherosclerosis, restenosis and other **smooth muscle** proliferative disorders, as well as chronic inflammation. In particular it is anticipated that mutation or deletion of both alleles of. . . occur due to de-differentiation of chondrocytes or osteocytes, as well as vascular disorders which involve de-differentiation of endothelial tissue and **smooth muscle** cells, gastric ulcers characterized by degenerative changes in glandular cells, and renal conditions marked by failure to differentiate, e.g. Wilm's. . .

DETD Any cell type or tissue, preferably monocytes, endothelial cells, or **smooth muscle** cells, in which the conservin is expressed may be utilized in the diagnostics described below. For example, a subject's bodily. . .

DETD . . . sequence, profiles may also be assessed in such detection schemes. Fingerprint profiles may be generated, for example, by utilizing a **differential display** procedure, Northern analysis and/or RT-PCR.

=> d bib ab 2

L4 ANSWER 2 OF 2 USPATFULL

AN 1998:111795 USPATFULL

TI Conservin nucleic acid molecules and compositions

IN Falb, Dean A., Wellesley, MA, United States
Gimeno, Carlos J., Boston, MA, United States

PA Millennium Pharmaceuticals, Inc., Cambridge, MA, United States (U.S.

corporation)
PI US 5807708 19980915
AI US 1996-688609 19960730 (8)
DT Utility
EXNAM Primary Examiner: Walsh, Stephen; Assistant Examiner: Kaufman, Claire M.
LREP Lahive & Cockfield, LLP
CLMN Number of Claims: 54
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 3939
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the discovery of novel conservin genes and polypeptides. Therapeutics, diagnostics and screening assays based on these molecules are also disclosed.

L10 ANSWER 17 OF 44 MEDLINE
AN 97470973 MEDLINE
DN 97470973
TI Integration of multiple instructive cues by neural crest stem cells reveals cell-intrinsic biases in relative growth factor responsiveness.
AU Shah N M; Anderson D J
CS Division of Biology 216-76, Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125, USA.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Oct 14) 94 (21) 11369-74.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199801
AB Growth factors can influence lineage determination of **neural crest** stem cells (NCSCs) in an instructive manner, in vitro. Because NCSCs are likely exposed to multiple signals in vivo, these findings raise the question of how stem cells would integrate such combined influences. Bone morphogenetic protein 2 (BMP2) **promotes** neuronal **differentiation** and glial growth factor 2 (GGF2) **promotes** glial **differentiation**; if NCSCs are exposed to saturating concentrations of both factors, BMP2 appears dominant. By contrast, if the cells are exposed to saturating concentrations of both BMP2 and transforming growth factor beta1 (which **promotes** smooth **muscle differentiation**), the two factors appear codominant. Sequential addition experiments indicate that NCSCs require 48-96 hrs in GGF2 before they commit to a glial fate, whereas the cells commit to a smooth **muscle** fate within 24 hr in transforming growth factor beta1. The delayed response to GGF2 does not reflect a lack of functional receptors; however, because the growth factor induces rapid mitogen-activated protein kinase phosphorylation in naive cells. Furthermore, GGF2 can attenuate induction of the neurogenic transcription factor mammalian achaete-scute homolog 1, by low doses of BMP2. This short-term antineurogenic influence of GGF2 is not sufficient for glial lineage commitment, however. These data imply that NCSCs exhibit cell-intrinsic biases in the timing and relative dosage sensitivity of their responses to instructive factors that influence the outcome of lineage decisions in the presence of multiple factors. The relative delay in glial lineage commitment, moreover, apparently reflects successive short-term and longer-term actions of GGF2. Such a delay may help to explain why glia normally **differentiate** after neurons, in vivo.
AB Growth factors can influence lineage determination of **neural crest** stem cells (NCSCs) in an instructive manner, in vitro. Because NCSCs are likely exposed to multiple signals in vivo, these findings raise the question of how stem cells would integrate such combined influences. Bone morphogenetic protein 2 (BMP2) **promotes** neuronal **differentiation** and glial growth factor 2 (GGF2) **promotes** glial **differentiation**; if NCSCs are exposed to saturating concentrations of both factors, BMP2 appears dominant. By contrast, if the cells are exposed to saturating concentrations of both BMP2 and transforming growth factor beta1 (which **promotes** smooth **muscle differentiation**), the two factors appear codominant. Sequential addition experiments indicate that NCSCs require 48-96 hrs in GGF2 before they commit to a glial fate, whereas the cells commit to a smooth **muscle** fate within 24 hr in transforming growth factor beta1. The delayed response to GGF2 does not reflect a lack of. . . moreover, apparently reflects successive short-term and longer-term actions of GGF2. Such a delay may help to explain why glia

normally **differentiate** after neurons, in vivo.

L10 ANSWER 19 OF 44 MEDLINE
AN 96427395 MEDLINE
DN 96427395
TI Smooth muscle lineage diversity in the chick embryo. Two types of aortic smooth muscle cell differ in growth and receptor-mediated transcriptional responses to transforming growth factor-beta.
AU Topouzis S; Majesky M W
CS Department of Pathology, Baylor College of Medicine, Houston, Texas 77030, USA. stavros@bcm.tmc.edu or. mmajesky@bcm.tmc.edu
NC HL-47655 (NHLBI)
SO DEVELOPMENTAL BIOLOGY, (1996 Sep 15) 178 (2) 430-45. Journal code: E7T. ISSN: 0012-1606.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199702
EW 19970204
AB Lineage analysis studies in the avian embryo have identified two types of smooth **muscle** cells (SMCs) in the tunica media of large elastic arteries; one that originates within the cardiac **neural crest** and is ectoderm in origin (Ect) and another that arises from local mesenchyme of mesodermal origin (Mes). To determine if differences in primary embryonic lineage can give rise to SMCs with stable differences in growth and **differentiation** properties, we isolated Ect and Mes SMCs from the Day 14 chick embryo aorta. We report that despite **different** primary embryonic origins, Ect and Mes SMCs **express** nearly identical levels of seven SMC **differentiation** markers in vitro, consistent with their common smooth **muscle** developmental fates in vivo. By contrast, Ect SMCs displayed a greater capacity for growth in serum-free medium than Mes SMCs, but only under conditions permitting short-range cell-cell interactions. Most of the peptide growth factors tested that might account for serum-independent growth (PDGF-AA, PDGF-BB, basic FGF, EGF, or activin) stimulated DNA synthesis to similar extents in Ect and Mes SMCs. However, we found dramatic, lineage-dependent differences in SMC responses to transforming growth factor-beta (TGF-beta). Exposure to TGF-beta 1 (0.4 to 400 pmole/liter) consistently increased DNA synthesis in Ect SMCs, whereas in paired cultures of Mes SMCs, TGF-beta 1 was growth **inhibitory**. In SMC cultures transfected with p3TP-lux, a luciferase reporter controlled by the TGF-beta 1-response elements of the human PAI-1 **promoter**, TGF-beta 1 (120 pM) produced 12 +/- 2-fold increases in luciferase activity in Ect SMCs and only 3 +/- 1.5-fold increases in Mes SMCs. Analysis of TGF-beta receptor phenotypes by Northern blot, radioligand binding, and crosslinking assays showed that Ect and Mes SMCs **expressed** similar levels of types I, II, and III TGF-beta receptors. However, using a polyclonal antibody specific for the chick type II TGF-beta receptor subunit, we demonstrate that Mes SMCs produce a fully glycosylated form of this protein while Ect SMCs elaborate only an unglycosylated type II TGF-beta receptor. These results show that Ect and Mes SMCs exhibit lineage-dependent differences in growth and receptor-mediated transcriptional responses to at least one important class of SMC morphogens and growth modifiers, e.g., the TGF-betas. Our

findings suggest that **different** SMC populations within a common vessel wall may respond in lineage-dependent ways to signals that direct formation of the tunica media in the embryo and to factors involved in the progression of vascular disease later in life.

AB Lineage analysis studies in the avian embryo have identified two types of smooth **muscle** cells (SMCs) in the tunica media of large elastic arteries; one that originates within the cardiac **neural crest** and is ectoderm in origin (Ect) and another that arises from local mesenchyme of mesodermal origin (Mes). To determine if differences in primary embryonic lineage can give rise to SMCs with stable differences in growth and **differentiation** properties, we isolated Ect and Mes SMCs from the Day 14 chick embryo aorta. We report that despite **different** primary embryonic origins, Ect and Mes SMCs **express** nearly identical levels of seven SMC **differentiation** markers in vitro, consistent with their common smooth **muscle** developmental fates in vivo. By contrast, Ect SMCs displayed a greater capacity for growth in serum-free medium than Mes SMCs,. . . 400 pmole/liter) consistently increased DNA synthesis in Ect SMCs, whereas in paired cultures of Mes SMCs, TGF-beta 1 was growth **inhibitory**. In SMC cultures transfected with p3TP-lux, a luciferase reporter controlled by the TGF-beta 1-response elements of the human PAI-1 **promoter**, TGF-beta 1 (120 pM) produced 12 +/- 2-fold increases in luciferase activity in Ect SMCs and only 3 +/- 1.5-fold. . . SMCs. Analysis of TGF-beta receptor phenotypes by Northern blot, radioligand binding, and crosslinking assays showed that Ect and Mes SMCs **expressed** similar levels of types I, II, and III TGF-beta receptors. However, using a polyclonal antibody specific for the chick type. . . responses to at least one important class of SMC morphogens and growth modifiers, e.g., the TGF-betas. Our findings suggest that **different** SMC populations within a common vessel wall may respond

L10 ANSWER 20 OF 44 MEDLINE
AN 96400084 MEDLINE
DN 96400084
TI Embryonic lineage of vascular smooth muscle cells determines responses to collagen matrices and integrin receptor expression.
AU Thieszen S L; Dalton M; Gadson P F; Patterson E; Rosenquist T H
CS Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha 68198-6395, USA.
SO EXPERIMENTAL CELL RESEARCH, (1996 Aug 25) 227 (1) 135-45.
Journal code: EPB. ISSN: 0014-4827.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199612
AB Developmental studies have demonstrated that the vascular smooth **muscle** cells (VSMC) present within the elastic arteries are **differentiated** from two definitive origins, the **neural crest** and the mesoderm. Cells from these distinct progenitors differ in their ability to determine long-range spatial order of the extracellular matrix, in proliferative responses, and in the **expression** of critical proteins. The present study utilizes collagen gel contraction assays and the analysis of integrin receptor subunit **expression** to evaluate cell-matrix interactions. In the presence of serum and transforming growth factor-beta 1 (TGF) or TGF-beta 1 alone, VSMC isolated from the abdominal aorta (AA-VSMC) were found to contract collagen matrices to a significantly greater extent than VSMC from the thoracic aorta (TA-VSMC). However, in TA-VSMC, beta 1 integrin and gel contraction were stimulated only in the presence of serum factors.
Metabolic labeling and immunoprecipitation of integrin subunits revealed that TGF-beta 1 induced beta 1 and alpha 5 integrin subunits in AA-VSMC four-and ninefold, respectively. AA-VSMC gel contraction stimulated by serum and TGF-beta 1 alone was **inhibited** with anti-beta 1 integrin antibody by 70 and 100%, respectively. However, the beta 1 integrin-specific antibody **inhibited** serum-induced TA-VSMC gel contraction by 25%. The data suggest that vascular smooth **muscle** cell ontogeny is an important determinant of cell function, phenotype, and response to growth factors such as TGF-beta 1.
AB Developmental studies have demonstrated that the vascular smooth **muscle** cells (VSMC) present within the elastic arteries are **differentiated** from two definitive origins, the **neural crest** and the mesoderm. Cells from these distinct progenitors differ in their ability to determine long-range spatial order of the extracellular matrix, in proliferative responses, and in the **expression** of critical proteins. The present study utilizes collagen gel contraction assays and the analysis of integrin receptor subunit **expression** to evaluate cell-matrix interactions. In the presence of serum and transforming growth factor-beta 1 (TGF) or TGF-beta 1 alone, VSMC. . . alpha 5 integrin subunits in AA-VSMC four-and ninefold, respectively. AA-VSMC gel contraction stimulated by serum and TGF-beta 1 alone was **inhibited** with anti-beta 1 integrin antibody by 70 and 100%, respectively. However, the beta 1 integrin-specific antibody **inhibited** serum-induced TA-VSMC gel contraction by 25%. The data suggest that vascular smooth **muscle** cell ontogeny is an important determinant of cell function, phenotype, and response to growth factors such as TGF-beta 1.

L10 ANSWER 23 OF 44 MEDLINE
AN 94176676 MEDLINE
DN 94176676
TI Backtransplantation of chick cardiac neural crest cells cultured in LIF rescues heart development.
AU Kirby M L; Kumiski D H; Myers T; Cerjan C; Mishima N
CS Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta 30912-2000..
NC HL36059 (NHLBI)
SO DEVELOPMENTAL DYNAMICS, (1993 Dec) 198 (4) 296-311.
Journal code: A9U. ISSN: 1058-8388.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199406
AB The cardiac **neural crest** is essential for normal development of the cardiovascular system. Cardiac **neural crest** cells are derived from the neural folds located between the mid-otic placodes and the caudal limit of somite 3. These crest cells can **differentiate** into a variety of mesenchymal cell types that support cardiovascular development, in addition to neurogenic cells. When cultured, many **express** alpha-smooth **muscle** actin or neurofilaments and lose their undifferentiated **neural crest** phenotype as shown by a decrease in HNK-1 reactivity. We wanted to determine whether cultured cardiac **neural crest** cells maintained the potency to support normal heart development when backtransplanted into embryos lacking their native cardiac **neural crest**. Under usual circumstances removal of the cardiac **neural crest** results in 80-100% incidence of persistent truncus arteriosus. The present study reports a system in which cardiac neural folds are cultured for 3 days and the cells backtransplanted into chick embryos after laser-induced ablation of the intrinsic cardiac neural folds. Rescue of heart development was improved 50% when cultured cells were backtransplanted and almost 200% when the backtransplanted cells had been cultured in leukemia **inhibitory** factor (LIF). To determine whether the cultured cells are capable of following normal migratory routes, cultured homospecific cardiac **neural crest** cells were tagged with DiI. Initially, fluorescent cells were found concentrated around the neural tube. By the second day following backtransplantation, the cells had migrated to the circumpharyngeal crest, populated the pharyngeal arches and aortic arch arteries, and were in the region of the cardiac outflow tract. By the third day, the labeled cells had dispersed, but could be found around the neural tube, esophagus, cardiac outflow tract, and within the dorsal root ganglia. Interestingly, a cranial migration to the periphery of the eyes was also noted. With the exception of the cranial migration to the eyes, cultured and backtransplanted cardiac **neural crest** cells followed normal migratory pathways to the cardiac outflow tract. LIF is used for the in vitro maintenance of the pluripotential phenotype of embryonic stem cells. In an effort to understand why LIF improves the ability of cultured **neural crest** cells to support normal heart development, we have examined the relationship of **neural crest expression** of HNK-1 antigen, alpha-smooth **muscle** actin, and neurofilament protein in **neural crest** cells cultured in LIF. LIF treatment resulted in an expanded period of **expression** of HNK-1 antigen, associated with a decrease in **expression** of alpha-smooth

muscle actin.(ABSTRACT TRUNCATED AT 400 WORDS)

AB The cardiac **neural crest** is essential for normal development of the cardiovascular system. Cardiac **neural crest** cells are derived from the neural folds located between the mid-otic placodes and the caudal limit of somite 3. These crest cells can **differentiate** into a variety of mesenchymal cell types that support cardiovascular development, in addition to neurogenic cells. When cultured, many **express** alpha-smooth **muscle** actin or neurofilaments and lose their undifferentiated **neural crest** phenotype as shown by a decrease in HNK-1 reactivity. We wanted to determine whether cultured cardiac **neural crest** cells maintained the potency to support normal heart development when backtransplanted into embryos lacking their native cardiac **neural crest**. Under usual circumstances removal of the cardiac **neural crest** results in 80-100% incidence of persistent truncus arteriosus. The present study reports a system in which cardiac neural folds are. . . was improved 50% when cultured cells were backtransplanted and almost 200% when the backtransplanted cells had been cultured in leukemia **inhibitory** factor (LIF). To determine whether the cultured cells are capable of following normal migratory routes, cultured homospecific cardiac **neural crest** cells were tagged with DiI. Initially, fluorescent cells were found concentrated around the neural tube. By the second day following. . . of the eyes was also noted. With the exception of the cranial migration to the eyes, cultured and backtransplanted cardiac **neural crest** cells followed normal migratory pathways to the cardiac outflow tract. LIF is used for the in vitro maintenance of the pluripotential phenotype of embryonic stem cells. In an effort to understand why LIF improves the ability of cultured **neural crest** cells to support normal heart development, we have examined the relationship of **neural crest** **expression** of HNK-1 antigen, alpha-smooth **muscle** actin, and neurofilament protein in **neural crest** cells cultured in LIF. LIF treatment resulted in an expanded period of **expression** of HNK-1 antigen, associated with a decrease in **expression** of alpha-smooth **muscle** actin.(ABSTRACT TRUNCATED AT 400 WORDS)

L6 ANSWER 11 OF 13 MEDLINE
AN 86298317 MEDLINE
DN 86298317 PubMed ID: 3742561
TI Morphological and functional **differentiation** of cultured **vascular smooth-muscle** cells.
AU Tagami M; Nara Y; Kubota A; Sunaga T; Maezawa H; Fujino H; Yamori Y
SO CELL AND TISSUE RESEARCH, (1986) 245 (2) 261-6.
Journal code: 0417625. ISSN: 0302-766X.
CY GERMANY, WEST: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198610
ED Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19861022
AB In numerous investigations using cultured smooth-muscle cells, investigators have consistently added 10-20% **fetal calf serum** (FCS) to the medium to maintain viable cells. In the present study we utilized an optical technique to investigate whether smooth-muscle cells, cultured with or without FCS, maintain their contractile activity in vitro. With such optical measurement, we were able to detect signals due to spontaneous contractions, in muscle cells cultured in FCS-free medium for up to 8 days, and, for the first time, were also able to observe the conduction of these cell contractions. The ultrastructural characteristics of cultured smooth-muscle cells during contractile activity, were also examined by electron microscopy. The cells were mature and well-differentiated, and were packed with numerous myofilaments. They had developed long cell processes, and were linked to one another by gap junctions. These observations indicated that the smooth-muscle cells, cultured without FCS for 7 to 8 days, were morphologically mature and maintained their contractile activity, whereas the cells cultured in FCS-containing medium showed no detectable signs of contractile activity.

L6 ANSWER 10 OF 13 MEDLINE
AN 92230777 MEDLINE
DN 92230777 PubMed ID: 1533095
TI Growth factors downregulate **vascular smooth muscle** thromboxane receptors independent of cell growth.
AU Dorn G W 2nd; Becker M W
CS Department of Medicine/Cardiology, University of Cincinnati College of Medicine, Ohio 45267.
SO AMERICAN JOURNAL OF PHYSIOLOGY, (1992 Apr) 262 (4 Pt 1) C927-33.
Journal code: 0370511. ISSN: 0002-9513.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199205
ED Entered STN: 19920607
Last Updated on STN: 19920607
Entered Medline: 19920521
AB Growth factors, in addition to being mitogenic, may modulate **vascular smooth muscle differentiation**. We tested whether serum or defined growth factors could regulate thromboxane A2 (TxA2) receptors in cultured rabbit aorta smooth muscle cells. **Fetal bovine serum** (10%) stimulated cell proliferation and DNA synthesis in subconfluent cell cultures. Binding of the thromboxane A2 agonist [1S-(1 alpha 2 beta(5Z),3 alpha(1E,3S),4 alpha)]-7-[3-(3-hydroxy-4-p- iodophenoxy-1-butenyl)-7-oxabicyclo[2.2.1]heptan-2-yl]-5-hep tenoic acid showed a 41% decrease in TxA2 receptors in cells treated with 10% serum compared with serum-deprived (0.1%) controls. Receptor downregulation by serum was gradually reversible upon serum withdrawal. Compared with serum-deprived cells, those exposed to 10% serum also had diminished TxA2-stimulated phosphatidylinositol hydrolysis. Regulatory actions of serum on TxA2 receptors were distinguished from mitogenic effects with heparin, which prevented cell growth but did not inhibit serum-induced downregulation of TxA2 receptors. Furthermore, low concentrations of platelet-derived growth factor and basic fibroblast growth factor decreased TxA2 receptors without stimulating cell proliferation or DNA synthesis. These observations describe a previously unrecognized regulatory action of growth factors on a **vascular smooth muscle** vasoconstrictor receptor, an action which is independent of effects on cell proliferation or DNA synthesis.

L3 ANSWER 1 OF 2 MEDLINE
AN 97460994 MEDLINE
DN 97460994 PubMed ID: 9315361
TI Characterization of molecular determinants of smooth muscle cell heterogeneity.
AU Halayko A J; Rector E; Stephens N L
CS Department of Physiology, University of Manitoba, Winnipeg, Canada.
SO CANADIAN JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY, (1997 Jul) 75 (7) 917-29.
Ref: 84
Journal code: 0372712. ISSN: 0008-4212.
CY Canada
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW LITERATURE)
LA English
FS Priority Journals
EM 199711
ED Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971105
AB Broad diversity in contractile and pharmacological properties of different smooth muscles is well recognized. Differences in proliferative capacity, electrophysiology, phenotypic marker protein content, matrix synthesis, and expression of cell-specific transcription factors between individual smooth muscle cells (SMCs) have also been reported. Precise developmental and molecular mechanisms underlying heterogeneity are not known; however, their elucidation is the thrust of much current research involving **vascular smooth muscle**. In contrast, limited studies of heterogeneity of subtypes of airway SMCs are available. In this report, we review molecular aspects of **differentiation** that may determine phenotypic heterogeneity of SMCs and also present data from our own studies characterizing heterogeneity in the proliferative capacity and marker protein content of airway SMCs. Using flow cytometry, cell cycle transit was monitored for cultured canine tracheal SMCs. Only 70% of arrested cells responded and traversed the cell cycle when stimulated with **10% fetal bovine serum**. Furthermore, heparin inhibited 40% of serum-responsive cells from entering the cell cycle, suggesting that both serum- and heparin-sensitive and -insensitive airway SMCs exist. Flow cytometric analysis of contractile protein and DNA content in freshly dissociated canine tracheal SMCs revealed that diploid (approximately 87%) and tetraploid (approximately 13%) populations exist. Clusters of SMCs having "high" or "low" smooth muscle myosin or alpha-actin content were also discerned, indicating that distinct subtypes of SMCs exist in mature airways. Diversity of SMCs may be a critical factor determining specific responses of smooth muscles to a number of physiological or pathophysiological stimuli that may include, for example, inflammatory mediators in asthmatic airways.